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Abstract

Title of the Thesis: Molecular Epidemiology of Epidemic Severe Malaria Caused by *Plasmodium vivax* in the State of Amazonas, Brazil.

Name: Patricia Dantas Santos-Ciminera, PhD in Emerging Infectious Diseases, 2005

Thesis directed by: Gerald V. Quinnan Jr. M.D., PMB

Malaria in South America is a major public health problem. In Brazil, most of the cases occur in the Amazon Region, particularly in the State of Amazonas. In Manaus, the capital of Amazonas, atypical cases of *P. vivax* infections, including patients presenting with severe thrombocytopenia and bleeding, led to the formulation of the hypothesis that severe disease could be related to a particular, emergent and more pathogenic genotype of *P. vivax*. We described the epidemiology of malaria for the Amazonas State and city of Manaus by comparing patients admitted in the hospital to those treated as outpatients in the Fundação de Medicina Tropical do Amazonas (FMT-AM). Admissions due to *vivax* malaria increased significantly from 1997 through 2003 suggesting a change in clinical presentation. The admitted group presented higher mean parasite counts, lower platelet counts, and higher levels of liver enzymes, higher total and indirect bilirubin, and higher blood urea nitrogen when compared to the outpatient group. Clinical symptoms of severe disease, including hematuria, hemolytic anemia, and thrombocytopenia were only noted in the admitted group. Furthermore, the presence of a palpable liver was more frequent in admitted patients. Nucleic acid sequences of three genes from *P. vivax*, the 18S SSUrRNA Type A gene, CSP gene and MSP-1 gene, were determined. Strains from test samples were compared to each other, to the reference strains Salvador I and Belém and to sequences retrieved from the Gene Bank. There were two main polymorphisms in the

18S SSUrRNA Type A gene, a cytosine/thymidine polymorphism at residue 100 of the alignment and a thymidine (T)/adenine (A) polymorphism at residue 117. Ten of eleven (10/11) admitted patients were 117:T compared to 13/21 outpatients. This frequency difference was statistically significant ($P<0.05$). The Salvador I strain was T at this position and the Belém strain was A. In the CSP gene, we identified 15 unique sequences of the VK210 strain; one sample had a mixed infection with *P. vivax*-like. The sources of variation in the CSP gene included the numbers of repeat segments, alanine/aspartic acid polymorphism at position five of a common repeat, and sporadic mutations. Frequent synonymous substitutions of the common repeat occurred in codons 1, 2 and 7, while the mutations at codon 5 were always non-synonymous. Among MSP-1 gene sequences, four recombination sites were distinguished between the interspecies conserved regions 5 and 6. Recombination among progenitor strains, closely related to the Salvador I and Belém strains, was the main source of diversity among the strains. The second most significant form of variation was in the polyglutamine region of strains with Belém-like sequences in the central part of this gene segment. There was no clustering of MSP-1 sequences in patients with severe disease. It was not possible to demonstrate the evolutionary relationship among our test samples by tests of phylogeny that incorporated sequence data for all three genes tested. The factors that may have limited the power of a combined analysis included small sample size and differences in the mechanisms and extent of variation among the genes. The retrospective study was unable to demonstrate that a particular strain of *P. vivax* was responsible for severe disease requiring hospitalization. This is the first detailed description of the genetic diversity among the *P. vivax* population in the Amazonas State of Brazil.

Molecular Epidemiology of Epidemic Severe Malaria Caused by *Plasmodium vivax* in the State of Amazonas, Brazil

By

Patricia Dantas Santos-Ciminera

Thesis submitted to the Faculty of the Emerging Infectious Diseases Program

Uniformed Services University of the Health Sciences

In partial fulfillment of the requirements for the degree of Philosophy Doctor 2005

To my beloved parents, João Secundo and Solange

My dear sisters, Andréa and Daniela

And to my more than loved husband, Paul

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Hypothesis and Specific Aims

In Brazil, malaria is largely restricted to the Amazon Region, and is particularly prevalent in the Amazonas State. Endemic transmission was reintroduced to urban areas including the periphery of Manaus, the capital of the State, in the late 1980s.

The reference center for diagnosis and treatment of malaria in the Amazonas State is the Fundação de Medicina Tropical do Amazonas (FMT-Am). Patients with fever are routinely screened for malaria, especially if they have history of travel to high-risk areas within the State. In the FMT-Am, clinicians have reported severe disease, with fatalities and drug resistance in patients with *Plasmodium vivax* malaria.

Effective prevention in this region depends on fully understanding the epidemiology of malaria. Accordingly, we evaluated the epidemic of malaria caused by *Plasmodium vivax* in the Amazon region of Brazil by studying patients diagnosed and treated in the Fundação de Medicina Tropical do Amazonas (FMT-Am).

The following primary and secondary hypotheses tested were:

Primary: The recent epidemic, including severe and relapsing malaria in Amazonas State, is due to the emergence of a highly pathogenic genetic variant of *P. vivax*.

Secondary: The recent epidemic, including severe and relapsing malaria in Amazonas State is due to the re-emergence of previously circulating variants of *P. vivax*, and reflects inadequacy of existing control measures.

To test these hypotheses, we pursued the following specific aims:

1. Describe the epidemiology of malaria in the Amazonas State and City of Manaus from 1980 to 2003.

2. Describe the phylogeny of *P. vivax* in patients diagnosed in the FMT-Am by studying partial sequences of genes coding for the 18S Small Subunit Ribosomal RNA (SSUrRNA) type A, Circumsporozoite protein (CSP) and Merozoite Surface Protein – 1 (MSP-1).

3. Determine if there are genetic differences in the three studied genes, between the strains circulating now compared to those circulating in the past, by comparing archived and current blood smear samples.

4. Determine if there are genetic differences in parasites found in patients with *P. vivax* malaria with severe or relapsing disease compared to patients with uncomplicated *P. vivax* malaria. Specifically, since the number of hospitalizations for *P. vivax* malaria has doubled, determine whether at least half of recently hospitalized patients are infected with a particular genotype.

5. Determine if genetic variant strains are associated with different laboratory outcomes of malaria caused by *P. vivax* treated in the FMT-Am.

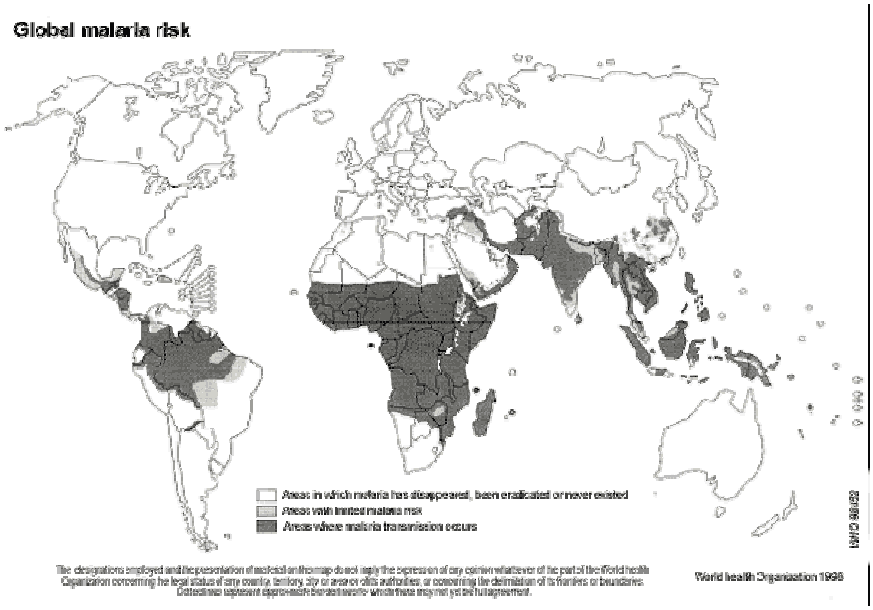
1. Background and Significance:

1.1 Malaria and its Current Epidemicity:

Human malaria is a disease caused by protozoan parasites of the genus *Plasmodium*. Four species (*P. falciparum, P. vivax*, *P. malariae* and *P. ovale*) cause human infections. These infections are associated with fevers that typically cycle with shaking chills and drenching sweats. The duration and severity of these cycles depends on the species infecting the person and the presence of prior immunity. Some malaria infections are asymptomatic (Gilles, 1996; Alves et al., 2002).

The World Health Organization estimates that more than 2.4 billion of the world's population are at risk of acquiring malaria in 100 countries or territories where malaria transmission occurs. This disease is mainly confined to the poorer tropical areas of Africa, Asia and Latin America (Figure 1; WHO, 2000).

Figure 1: Global distribution of Malarious areas, WHO (2000)

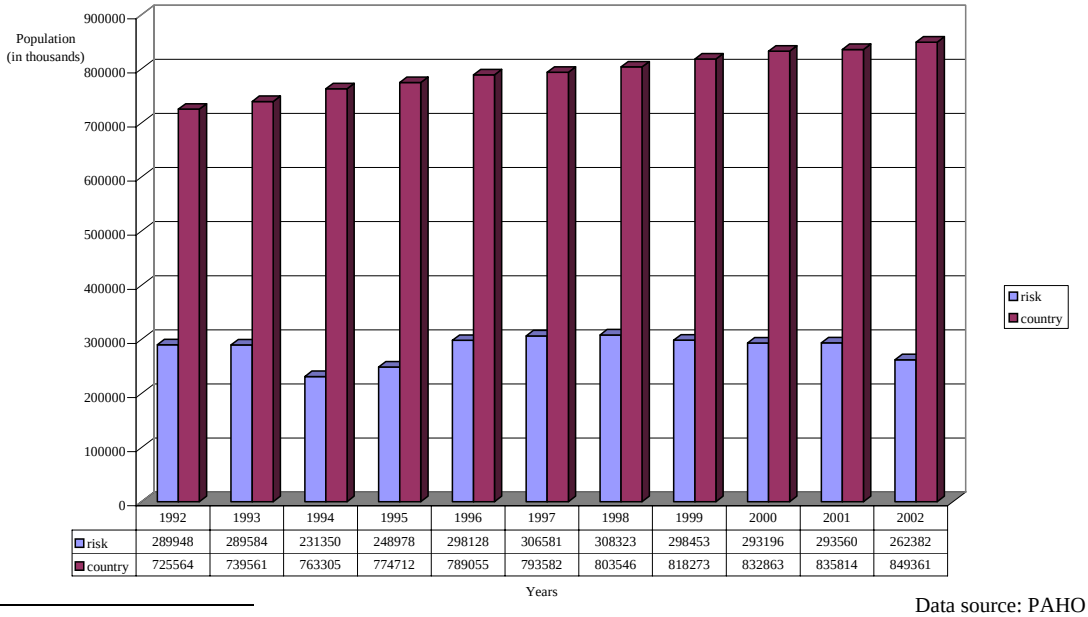


WHO

The incidence of malaria worldwide is estimated to be between 300 - 500 million clinical cases annually; of these, 1.1 – 2.7 million die every year, mainly children under the age of 5 in sub-Saharan Africa. Fatal infections are usually caused by *Plasmodium falciparum* (WHO, 2000), although *Plasmodium vivax* causes a significant burden in those countries where it is prevalent (Mendis et al. 2001), until recently there were few recognized cases of life threatening or fatal malaria caused by *P. vivax*.

According to the Pan American Health Organization, the total population at ecological risk* of malaria transmission in the countries of the Americas, in which malaria was present, was 262 million in 2002, representing 30.86% of a total population of 849 million. This percentage has not undergone significant change since 1992 (Figure 2; PAHO, 2001 - 2003).

Figure 2: Population in areas with ecological risk of malaria transmission in the Americas, 1992-2002.



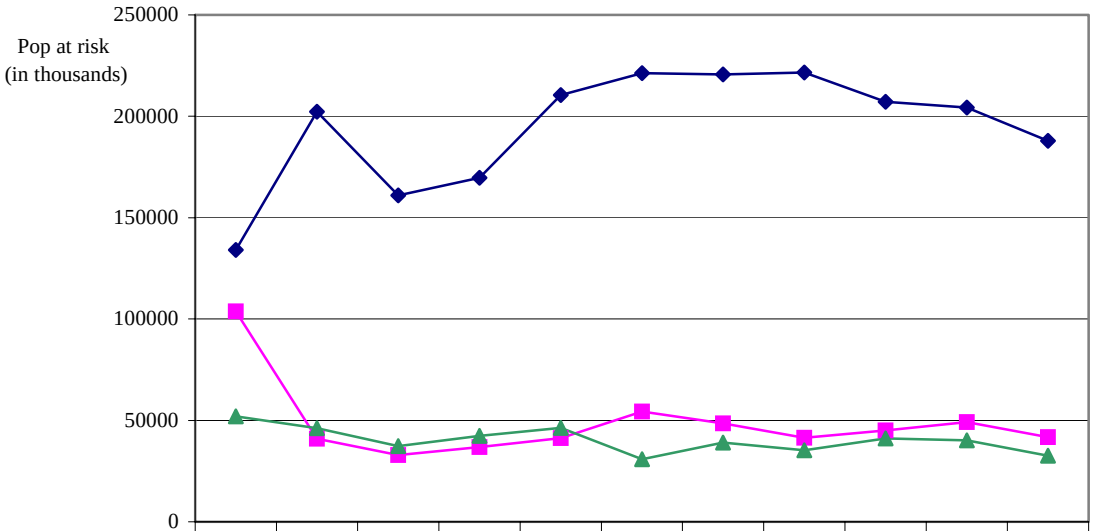
	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
risk	289948	289584	231350	248978	298128	306581	308323	298453	293196	293560	262382
country	725564	739561	763305	774712	789055	793582	803546	818273	832863	835814	849361

Data source: PAHO
Risk = Total Pop. at ecological risk
Country= Total pop. of countries with eco. risk

* Including countries with no active malaria transmission

The transmission risk for this population is classified as low, moderate or high based on the Annual Parasite Index (API=number of malaria cases/1000 persons)*. From Figure 3 we can observe that the areas with low ecological risk are the ones that have suffered major fluctuations since 1992. Areas with moderate risk had an initial decrease but have since remained constant, as have the areas of high risk for malaria transmission (PAHO, 2001-2003).

Figure 3: Malaria transmission risk for the population living in areas with ecological risk of malaria transmission, 1992-2002.



Risk	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
low	134089	202329	160974	169643	210519	221341	220702	221680	207099	204307	187972
moderate	103885	41030	32967	36881	41332	54358	48537	41444	44999	49124	41814
high	51974	46225	37409	42454	46277	30882	39084	35329	41098	40129	32596

Data source: PAHO

* API for most countries: Low risk, 1/1000, Mod risk 1/1000>API<10/1000, High risk API>10/1000. For Brazil: Low risk <10, Mod.risk 10>API<50, High risk API>50.

Of the 35 member countries of PAHO, there are 21 in which malaria transmission occurs. Eleven of these countries are in South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guyana, Guyana, Paraguay, Peru, Suriname and Venezuela. The remaining countries are in Central America: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama; and in North America: Mexico. The Andean region (Bolivia, Colombia, Ecuador, Peru and Venezuela) and Brazil account for more than 80% of the total cases of malaria registered in the Americas. From 1999 to 2003, the incidence of malaria increased noticeably in this area. In 2002, Colombia and Venezuela had increases in numbers of cases of 8% and 47%, respectively. The Andean region had an increase in malaria transmission from 32.3% in 1999 to 46.6% in 2002. However, Brazil decreased from 50.5% to 39.6% (PAHO, 2001-2003). Nine states in Brazil are reported as areas of very high (API≥50/1000) and high (API≥10/1000 up to 49.9/1000) risk of malaria transmission. All these highly endemic States are located in the North Region (PAHO, 2003).

The Malaria Eradication Campaign (CEM) established in Brazil in 1965 was based on the eradication strategies of the WHO. This campaign was able to eliminate malaria from extensive areas of the Brazilian territory but not from the Amazon Region (Loiola et al., 2002). Since the eradication campaign was abandoned, different strategies have been used in an attempt to minimize the incidence of malaria in the Amazon. Between the years 2000 and 2002, the Action Plan for Intensification of Control Measures in the Amazon (PIACM) was introduced as part of efforts to Roll Back Malaria (MS-SVS, 2003).

The Brazilian Ministry of Health reported that between January and August 2003 there was a 2.64% reduction in the number of cases (320,200) as compared with the same period in 2002 (328,881). However, this did not represent a uniform reduction in the number of malaria infections in the country. The States of Amazonas, Rondônia and Tocantins reported increases of 82.97%, 14.75% and 10.35% respectively (MS-SVS, 2003).

In 2003, the National Program for Malaria Control - PNCM (MS-SVS, 2003) proposed measures for the reduction of cases in the Amazon region and prevention of cases in States where transmission has been interrupted. The objectives of the PNCM included improvement of human resources for the early diagnosis and treatment of malaria, promoting health education and the application of selective control measures. We are unable to discuss the results of the new program because the numbers for 2004 are not yet available.

1.2 Identifying a Problem:

The Fundação de Medicina Tropical do Amazonas (FMT-Am) is located in the city of Manaus, Amazonas State, Brazil. Patients come to FMT-Am from Manaus and other municipalities in the state and are treated as outpatients in the malaria clinic or admitted to the hospital.

Recently the occurrence of atypical clinical cases of *P.vivax* with relapse of infection has been recognized at the FMT-Am. Alecrim (2000) described patients with malaria, concluding that severe disease, including thrombocytopenia with hemorrhagic manifestations, does occur during infection with *P. vivax*. In that series, 46 of 75 (61.3%)

patients admitted for treatment in the hospital were classified as having severe disease. Recently a case of immune thrombocytopenic purpura in a patient with *P. vivax* infection was reported (Lacerda et al., 2004). The patient's platelet count reached as low as $1x10^9$/L, and the patient presented with petechiae and bleeding. The thrombocytopenia was more severe than cases previously described in the literature (Anstey et al., 1992; Yamaguchi et al., 1997; Kakar et al., 1999). Thrombocytopenia is a well-known manifestation of malaria, but profound thrombocytopenia is usually associated with *Plasmodium falciparum* infections.

These observations have engendered the impression that *Plasmodium vivax* is causing more severe disease than previously recognized by clinicians at FMT-Am. Various possible explanations for the apparent changes in the clinical epidemiology of *P. vivax* malaria in Amazonas have been published (Alecrim, 2000). One possible explanation for more severe disease is that change in age-specific infections and increased infection rates over time may have resulted in more frequent occurrence of manifestations that were previously rare or unrecognized. An alternative possible explanation is that a genetic variant of *P. vivax* with greater virulence has recently emerged. A recent report lends support to the latter hypothesis. A possible clonal expansion in the variable region of the SSUrRNA gene was found in an isolate from a patient exhibiting chloroquine and primaquine resistance (Alecrim et al., 1999). A molecular epidemiological approach should clarify which of these possibilities best explains the recent observations.

1.3 The Standard of Care at the Fundacão de Medicina Tropical do Amazonas:

Patient diagnosis and management:

The endemicity of malaria in the Amazonas State led the FMT-Am to develop a standard of care for diagnosis and management of the patient with malaria. The following is an abstract of the guidelines provided by that institution (http://www.fmt.am.gov.br).

All patients with undifferentiated fever or coming from areas where malaria is endemic are screened for *Plasmodium* parasites using the thick smear method. The results are provided using the plus system, which entails using a code of between one and four plus signs: (+), when 1 to 10 parasites are found per 100 thick film fields; (++), when 11 to 100 parasites are found per 100 thick film fields; (+++), when 1 to 10 parasites are observed in a single thick film field; and, (++++) when more than ten parasites per single thick film field are found in the smear.

Patients with parasite levels equal to or above +++F or +++V, or any *P. falciparum* slide with the presence of schizonts are sent to the Emergency Room and are given priority treatment.

All patients with positive malaria results are evaluated by the attending physician and are treated as outpatients in the malaria clinic or are admitted to the hospital, depending on the clinical and laboratory results.

When the attending physician evaluates a patient, there are laboratory and clinical criteria for admission and treatment in the hospital. If the attending is not a malaria specialist and has doubts, he must contact the malaria staff to determine appropriate management. All patients that fit the laboratory and/or clinical profile for severe disease,

or have a condition that is likely to evolve to a severe status, are sent to the hospital unit for admission and treatment.

The laboratory profile specifications for admission to the hospital for treatment include: parasite count equal to or higher than +++F or +++V; the finding on thick smear of *P. falciparum* with the presence of schizonts; hypoglycemia; creatinine levels above 1.5 mg/dL; transaminase levels three times the normal level; total bilirubin above 4.0 mg/dL; hematocrit below 21% in adults and below 15% in children; radiologic signs of condensation or diffused infiltrate; and, platelet levels below 40,000/mm^3.

The clinical profile specifications for admission to the hospital are: generally debilitated state, pregnant, age less than 3 months, previous splenectomy, diabetes, hypertension, AIDS or another immunodeficiency disease, intense jaundice, oliguria, anuria, bleeding, disorientation, convulsion, hypotension, dyspnea, extremely pale gums, or intense abdominal pain.

A technician from the malaria laboratory makes a morning visit each day to the hospital to collect blood smears for parasite count.

Standard treatment for malaria in the FMT-Am:

All malaria caused by *Plasmodium vivax* is treated with chloroquine and primaquine. Adults with *P. falciparum* levels no higher than ++ are treated with quinine and doxycycline; pregnant women and children are treated with mefloquine or clindamycin, alone or in combination with quinine. For severe disease caused by *P. falciparum* or in the presence of peripheral schizonts, patients are treated with artesunate (IV) or artemether (IM) combined with mefloquine or clindamycin. Primaquine is added

for the treatment of mixed infections (*P.f + P.v*), when parasite levels of *P. falciparum* are equal to or greater than +++, or when gametocytes are present.

1.4 The Alecrim Cohort:

Alecrim (2000) conducted a descriptive study of patients attending FMT-Am for diagnosis and treatment of *Plasmodium vivax* malaria between June 1997 and July 1999. The sample size for that cohort was based on 6000 cases malaria diagnosed at the foundation in 1996.

Malaria was diagnosed by thick smear. When a high parasitemia was observed the ParaSight F® test was used to rule out mixed infection with *P. falciparum*.

An interview to obtain epidemiological data was conducted after diagnosis. The purpose of the project was explained and patients were invited to participate. Non-severe patients willing to participate were followed in the malaria clinic as outpatients. Those considered severe were admitted to the hospital. Continuation of treatment was ensured for those not in the project.

Inclusion criteria for patients followed in the outpatient malaria clinic were that the patient planned to stay in the urban area of Manaus during treatment (away from transmission risk) and during the follow-up period, and was age 12 years or older. Children under age of 12 and pregnant women were exceptionally included in the cohort when presenting with *P. vivax* malaria resistant to drugs or recrudescence. Ambulatory patients were excluded if they lived in areas of active malaria transmission or had frequent trips to these places, were pregnant, or had chronic diseases, including collagen

vascular diseases, chronic renal insufficiency, colitis, gastric ulcer, idiopathic thrombocytopenia, respiratory insufficiency, leukemia, and sickle cell anemia.

Patients admitted to the hospital were included in the study if they presented with *vivax* malaria classified as severe, or moderate with potential to become severe, and were excluded if they had co-morbid chronic diseases such as collagen vascular diseases, chronic renal insufficiency, colitis, gastric ulcer, idiopathic thrombocytopenia, respiratory insufficiency, leukemia, and sickle cell anemia. At their first visit, patients were classified as having mild, moderate or severe disease based on the following signs and symptoms defined by Alecrim (2000), as follows.

Patients were considered to have mild disease if they exhibited fever and axillary temperature equal to or less than 37.5^{o}C, or asthenia or myalgia. Moderate malaria was defined as patients with classic signs and symptoms of malaria including chills, fever, headache, nausea, vomiting, hepatomegaly and/or splenomegaly, and anemia.

Patients were considered severe if they showed classic signs and symptoms of malaria plus one or more of the following complications: jaundice with total bilirubin equal or higher than 5mg/dL, anemia with hemoglobin below 6mg/dL and hematocrit equal or below 20%, convulsive crisis, hypoglycemia, platelets below 49,000, bleeding, coma, shock, hemoglobinuric fever, spleen rupture, pulmonary alterations.

Patients with non-severe *P. vivax* malaria were treated and followed in the outpatient clinic. Those presenting clinical manifestations of severe disease were referred to the Emergency Room and admitted to the hospital for treatment. In the malaria outpatient clinic, clinical and epidemiological history was collected. Patients were questioned about travels and place of residence to define the probable origins of

infection, numbers of previously diagnosed malaria infections, and use of anti-malarial drugs. Physical examination was performed and vital signs collected. The follow-up visits were scheduled for days 3, 5, 7, 14, 21, 28, 35, 45, 60, 75 and 90 following the initial visit.

Patients with severe disease, or moderate disease tending to become severe, were followed from five to seven days in the hospital. Venous blood was collected from all patients. Hematological and biochemical tests were performed by automated methods using Coulter STK and Dade-Behring Dimension, respectively.

Anemia was defined as hemoglobin equal or less than 11g/dL and severe anemia when values reached 6g/dL or less. Thrombocytopenia and severe thrombocytopenia were defined as platelet counts less than 150,000/mm^3 and 50,000/mm^3, respectively. Alecrim (2000) classified parasite levels based on trophozoite counts as low (up to 8,000 trophozoites/mm3), medium (8,001-16,000 trophozoites/mm3) or high (above 16,000 trophozoites/mm3). Resistance to chloroquine was detected in 2.4% of the patients. Primaquine sensitivity was monitored in 141 patients followed for 90 days. It was observed that 28.4% of the patients presented relapse, assuming compliance.

A total of 426 patients were included in the cohort, 351 followed in the outpatient clinic and 75 admitted to the hospital. The main complications associated with hospitalization were thrombocytopenia and anemia. Severe thrombocytopenia was diagnosed in 50% of the admitted patients. Of those, 8% had bleeding, 4% had thrombocytopenic purpura and hemorrhage of conjunctiva, and 2.7% had hematuria.

The conclusions were that severe *P. vivax* malaria in the Amazonas State was associated with hemorrhagic manifestations and thrombocytopenia, and that there was resistance to both chloroquine and primaquine.

Plasmodium vivax variants VK210 and VK247 were detected using the technique of PCR followed by hybridization; *P. vivax*-like was not tested. *P. vivax* VK247 was only detected in mixed infections with VK210.

1.5 *Plasmodium vivax*:

1.5.1 Classification and Life Cycle:

Malaria parasites are members of the order *Coccidia*, sub-order *Haemosporidiidea*, family *Plasmodiidae*, genus *Plasmodium* (Gilles, 1996). Four species affect humans: *P. falciparum*, *P. vivax, P. malariae* and *P. ovale*. For this thesis we will be focusing on the species *Plasmodium vivax*

The cycle of all malaria parasites starts with the female of the *Anopheles* mosquito taking a blood meal containing sexual forms, gametocytes, of the parasite. The "male" gametocyte undergoes exflagellation producing "male" gametes or microgametes. The "female" gametocyte matures into the "female" gamete or macrogamete. Fertilization occurs in the stomach of the mosquito producing a zygote. The *P. vivax* sexual cycle takes 16 days at 20^{o}C and between 8-10 days at 28^{o}C (Gilles, 1996, Taylor & Strickland, 2000). After morphological changes, the oökinete is formed. In this stage the parasite crosses the stomach wall of the mosquito and changes its morphology into

the oöcyst (Gilles, 1996). Oöcysts nuclei divide and the sporozoites multiply inside it, increasing in number until its rupture. Released sporozoites migrate to the salivary gland of the vector host and are injected into the human host during a blood meal (Gilles, 1996).

Phagocytes destroy some sporozoites shortly after injection into the blood stream. The ones that survive reach the liver. They invade hepatocytes and undergo asexual multiplication. *Plasmodium vivax* pre-erythrocytic schizogony in humans lasts 6-8 days; during this period parasites differentiate into primary tissue schizonts or hypnozoites. Hypnozoites may stay dormant in the liver for weeks, months, or years. These forms can be responsible for relapses of infection. The trigger for relapse is still controversial (Gilles, 1996, Taylor & Strickland, 2000).

When schizonts reach maturity they burst, releasing about 10,000 merozoites into the bloodstream. The merozoite invades red blood cells by interaction with glycophorins present on the erythrocyte surface and is aided by their internal organelles, rhoptries and micronemes. Inside the red cell, the organism lives within a parasitophorous vacuole formed by invagination of the red cell membrane. *Plasmodium vivax* only invades reticulocytes and all asexual forms can be found in the peripheral blood (Gilles, 1996, Taylor & Strickland, 2000).

In the red blood cells (RBCs), young trophozoites are small and ring shaped. During maturation they assume an irregular ameboid form. Trophozoites later divide asexually in a process of erythrocytic schizogony. Merozoites are produced inside the schizonts. The erythrocyte ruptures in the end of schizogony and liberates merozoites that

have to invade new RBCs. The erythrocytic cycle lasts 42-48 hours, characterizing the periodicity of *P. vivax* fever (Gilles, 1996, Taylor & Strickland, 2000).

Subpopulations of merozoites differentiate into sexual gametocytes. Female macrogametocytes and male microgametocytes usually appear within 3 days. Upon feeding on humans, mosquitoes ingest gametocytes along with their blood meal. The sexual cycle of parasite reproduction is then completed within the mosquito (Gilles, 1996, Taylor & Strickland, 2000).

The complex life cycle of the malaria parasites is reflected in the number of proteins differentially expressed during each phase. Some of them are vaccine candidates due to their ability to stimulate immune responses that correlate with protection from infection or disease.

1.5.2 Clinical Manifestations of the Infection:

During *Plasmodium vivax* infection the prepatent period is between 11-13 days. The incubation period is 13 days, ranging from 12-17, but can be longer for some strains. The primary attack can be mild to severe. The periodicity of febrile attacks is 48 hours and the attacks last between 8-12 hours (Gilles, 1996, Taylor & Strickland, 2000).

Early in the infection, fever periodicity can be erratic or continuous. After five to 7 days of continued symptoms, synchrony may develop, with fever presenting approximately every 48 hours (Boulos, 1990; Gilles, 1996; Taylor & Strickland, 2000).

Headache, fever, body pain, prostration and nausea are symptoms reported during the primary attack. Paroxysms are complete periods of cold, heat and sweating, occurring in the afternoon, or less frequently, in the morning. If relapse of infection occurs due to

activation of hypnozoites, symptoms are mild or absent. Other clinical manifestations include enlarged and palpable spleen and liver (Boulos, 1990, Taylor & Strickland, 2000).

The signs, symptoms and complications experienced during malaria infection are related to the development of the parasite in the erythrocytes and destruction of the erythrocyte. The mechanical and metabolic effects of the *Plasmodium* result in microvascular disease with a strong metabolic component. The events following RBC rupture range from the classic paroxysms of malaria to more severe manifestations. Cytokines and adhesion molecules also play a role in the pathophysiology of malaria (Krogstad, 1995; White, 1998, Taylor & Strickland, 2000; Wickramashinghe & Abdalla, 2000).

During *Plasmodium vivax* infection, anemia is usually normochromic and normocytic and may be moderate to severe. Neutropenia, thrombocytopenia, lymphopenia and monocytosis may occur (Wickramashinghe & Abdalla, 2000). A case of haemophagocytic syndrome causing pancytopenia was described in a 41-year-old woman who acquired *P. vivax* infection in Costa Rica (Aouba et al., 2000).

Severe thrombocytopenia is described as a rare event in *vivax* malaria (White, 1998; Wickramashinghe & Abdalla, 2000). *Plasmodium vivax* parasites were observed intact inside platelets, and thrombocyte invasion was suggested (Fajardo & Tallent, 1974).

There are case-reports of profound thrombocytopenia in association with *vivax* infection in indigenous populations and in cases imported from various geographic regions where there is transmission of *P. vivax* (Anstey, Currie, Dyer, 1992; Horstmann

et al., 1981; Kakar et al., 1999; Kelton et al., 1983; Lacerda et al., 2004; Looareesuwan et al., 1992; Makkar et al., 2002; Yamaguchi et al., 1997). Oh and collaborators (2001) described severe thrombocytopenia with a platelet count <60,000/μL in 29.7% of patients (30 of 101) with symptomatic vivax malaria from Korea.

In cases of profound thrombocytopenia, correlation with high counts of parasites (Horstmann et al., 1981), association with anemia and leukopenia (Kakar et al., 1999), bleeding (Makkar et al., 2002; Lacerda et al., 2004) and purpura (Lacerda et al., 2004) have been described during *P. vivax* infection. Some authors have associated this event with immune-mediated mechanisms (Kelton et al., 1983, Yamaguchi et al., 1997) while others have not (Looareesuwan et al., 1992).

Other severe clinical manifestations were reported in cases of *P. vivax* infection. Noncardiogenic pulmonary edema (Curlin et al. 1999) and acute respiratory distress syndrome (Tanios et al., 2001) were present in imported cases of malaria.

A 22-year-old man presented with retinal hemorrhage associated with heavy parasitemia due to *P. vivax* in the scenario of reintroduced malaria in Korea. The patient was anemic but had platelet count of 145,000/mm^3 and normal levels of glucose and liver enzymes (Choi et al., 2004). Choi and colleagues (2004) reviewed the literature and found four other cases of retinal hemorrhage arising from *P. vivax* infections, all occurring in Asia (India and Korea). The patients were young and anemic. Normal platelet levels occurred in two. No conclusive mechanism was described.

Neurological manifestations are frequently described in children with *P. falciparum* infection in Africa (Boulos, 1990, Gilles, 1996, Krogstad, 1995; White, 1998, Taylor & Strickland, 2000). There are confirmed (Beg et al., 2002, Chakravarty et al.,

2004) and suspected (Braga et al., 2004) cases of cerebral and/or neurological involvement in patients with *vivax* malaria in the literature. Seizures, multiple basal ganglia infarcts, disorientation, delirium, torpor, prostration, acute inflammatory demyelinating polyneuropathy and Guillain Barré Syndrome were present in patients from various countries including Brazil.

1.5.3 Genetic Characteristics:

Plasmodium parasites are eukaryotes with nuclear genome organized in chromosomes and two extra-nuclear DNA elements, one mitochondrial and one apicoplast type. Malaria parasites are haploid during most of the life cycle; the zygote is the only diploid stage (Walliker et al., 1998).

A genome size of approximately 35-40 Mb for *P. ovale*, *P. vivax* and *P. malariae* was reported in 1999. All the species appeared to contain 14 chromosomes. The authors found synteny between the four human malaria parasites to be conserved 80% of the time, despite size differences of their chromosomes (Carlton et al., 1999). *Plasmodium falciparum* clone 3D7 nuclear genome is composed of 22.8 mega-bases (Mb) distributed in 14 chromosomes, with an overall 80.6% A+T content (Gardner et al., 2002). The Salvador I strain of *P. vivax* isolated in the 1960s from a natural infection in a patient from La Paz region of El Salvador is being sequenced using whole genome shotgun strategy (Carlton, 2003). The estimated 30 Mb genome is distributed among 14 chromosomes with approximate G+C content 45% and fewer poly (A) and poly (T) regions in relation to *P. falciparum*.

In a large-scale, multigene survey of variation within the genome, Feng et al. (2003) sequenced 100,924 bases of DNA from a 200-kilobase contiguous DNA sequence of one *P. vivax* chromosome from four laboratory-adapted lines originating in different geographical regions: India, El Salvador, Brazil and Thailand. A highly polymorphic genome was described. The authors found that single nucleotide polymorphisms clustered in intergenic regions and in specific genes, possibly under selective pressure.

Analysis of genetic variability at 13 microsatellite loci in 108 samples from 8 localities in various parts of the world revealed limited polymorphism of *P. vivax* (Lecrerc et al., 2004). This study placed the parasite in a monophyletic group with *Plasmodium* species parasitizing Old World Monkeys. The authors concluded that a selective sweep and/or population bottleneck occurred in a recent evolutionary past. *Plasmodium vivax* and *P. simium* share alleles at all 13 microsatellites. Based on polymorphism of the 18S ribosomal RNA gene and the 35 kilobase plastid genome the American and Asian/African *P.vivax* were reported by Li et al. (2001) to be distinct populations. In this study *Plasmodium simium* grouped with populations of Africa and Asia. Carter (2003) concludes that changes in the land topology and movement of primates were responsible for the separation of *P. vivax* and *P. simium*. The author hypothesizes that somewhere on the united African and Eurasian landmass is the place where these species diverged or originated.

Cui and collaborators (2003) argue that to study the genetic diversity of *Plasmodium vivax* is a crucial step to understand changes in clinical manifestations and resistance to anti-malarial drugs, and to comprehend parasite population structure that directly affect local and global epidemiology.

Among the many genetic markers of *P. vivax*, we chose to study the genetic diversity in the Amazonas State of Brazil by analyzing sequences of the 18S Small Sub Unit ribosomal RNA (18S SSUrRNA) Type A gene, since it has been hypothesized that a possible clonal expansion was associated with relapse/recrudescence of disease (Alecrim et al., 1999). In addition, we chose two protein-encoding genes that are vaccine candidates, Merozoite Surface Protein (MSP) and Circumsporozoite Protein (CSP) genes. These genes were chosen because the proteins are immunogenic and the genes are polymorphic.

From the various MSP genes described for *P. vivax*, we chose MSP-1, specifically the regions between the interspecies conserved blocks (ICBs) 5 and 6 of MSP-1. This region is dimorphic, and intragenic recombination has been suggested as a mechanism generating antigenic diversity (Porto et al., 1992; Premawansa et al., 1993, Mancilla et al., 1994; Kolakovich et al., 1996; Putaporntip et al., 1997). Isolates from Brazil (Porto et al., 1992) have been reported in the literature, but there is no information on the genetic diversity of MSP-1 sequences between ICB 5 and 6 from the Amazonas State.

Since the sporozoite is immunogenic and capable of eliciting immune protection, the Circumsporozoite Protein is the prime target of infection blocking vaccines. All malaria parasites have a single copy of this gene encoding a CSP with similar structure, a central variable region of tandem repeats flanked by nonrepeated conserved sequences (Marsh, 1996, Nardin & Zavala, 1998, Cui et al., 2003). Studies of *P. vivax* CSP distributed all over the world revealed variants based on the repeat region (Rosemberg et al., 1985; Arnot et al., 1990; Qari et al., 1992 and 1993a, 1993b). There is no report on

the diversity of the CSP gene in the Amazonas State of Brazil. There are a limited number of polymorphic markers available for *P. vivax* (Cui et al., 2003).

1.5.3.1 The Small Sub Unit Ribosomal Ribonucleic Acid (SSUrRNA) gene:

Plasmodium parasites have a low number of nuclear-encoded rRNA genes (between 4 to 8 per haploid genome) interspersed among other genes (McCutchan, 1986). Ribosomal RNA genes are present in three types 18S, 5.8S and 28S with 5-40% sequence diversity. These genes are expressed differentially based on stage of parasite development (Rogers et al., 1998). Transcription of each of the genes encoding the Small Sub Unit (18S) rRNA in *Plasmodium vivax* occurs distinctively during parasite development. Type A is expressed during asexual replication (erythrocytic schizogony); type O is expressed in the oökinete/oöcyst development in the mosquito, and the type S is expressed in oöcyst sporozoites and salivary gland sporozoites. These 18S rRNA genes are typically eukaryote-like in structure and possess extended variable region present in other malaria parasites (Li et al., 1994 and 1997).

Parasite Identification:

Comparisons between *Plasmodium* parasites demonstrated that the 18S SSUrRNA is composed of conserved and variable regions. This sequence diversity is the diagnostic target underlying various protocols used to identify and differentiate the four species that cause malaria in humans (Waters & McCutchan, 1989; Goman et al., 1991; Snounou et al., 1993a, 1993b, 1993c; Das et al., 1995; Li et al., 1995; Kawamoto et al., 1996; Tahar & Basco, 1997).

The use of molecular tools based on detection of the 18S SSUrRNA gene have proven useful in increasing the sensitivity and specificity of malaria diagnosis, and has demonstrated higher incidence of mixed infections than that demonstrated using the traditional diagnostic technique of the blood smear (Snounou et al., 1993a, 1993b). The same molecular technique has been used to identify malaria in vectors (Snounou et al., 1993c, Santos-Ciminera et al., 2004).

It is important to keep in mind that mutations altering the target region can lead to false negative results. Kawamoto et al. (1996) reported sequence variation in the 18SrRNA gene of two *P. ovale* isolates that resulted in failure to hybridize with a species-specific probe, and Liu et al. (1998) reported sequence variation in the gene of *P. malariae* isolates.

Genetic diversity:

Variation in the 18S rRNA gene is commonly used to make phylogenetic inferences between malaria parasites (Qari et al., 1996; Escalante et al., 1997; Rathore et al., 2001) and within a single species (Alecrim et al., 1999; Li et al., 2001). As mentioned previously the origin and evolution of malaria parasites is still controversial and is not the scope of this thesis. Together with other authors (Alecrim et al., 1999; Li et al., 2001) our interest was to study the genetic diversity of *Plasmodium vivax* and describe the diversity found in isolates from the Amazonas State, Brazil.

Alecrim et al. (1999) amplified and sequenced a segment of the SSUrRNA gene in samples from primary, secondary and tertiary infections in a patient from Manaus. The primary and following infections at days 35 and 180 of follow-up were identical to each other but four nucleotide substitutions were noted when these sequences were aligned

with isolates from Thailand and El Salvador. The authors suggested that a rare subset of the *P. vivax* population is disease-associated and maintains a degree of genetic isolation based upon sequence diversity.

Li et al. (2001) examined geographically distinct *P. vivax* isolates and tested their infectivity in *Anopheles albimanus*. They found that strains from the New World are similar to each other and distinct, as a group, from isolates from Asia and Oceania. The authors suggested a new nomenclature for *P. vivax* based on sequence diversity of the SSUrRNA gene.

1.5.3.2 The Circumsporozoite Surface Protein (CSP) gene:

The expression of CS protein occurs in the oöcyst and seems to play a role in the morphogenesis of the sporozoite. During migration to the salivary gland, shedding of the protein occurs. CSP is important for motility of the malaria parasite and for invasion of the vertebrate host's liver cells (Beier & Vanderberg, 1998; Frevert & Crisanti, 1998). Knockout of CSP gene in the primate malaria parasite *P. knowlesi* demonstrated that this protein is critical for sporozoite development (Kocken et al., 2002).

The molecular structure of CSP includes conserved amino and carboxy terminal regions flanking a variable repeat region. The variable repeats region consists of amino acid blocks that vary in number and sequence. Conserved region I is amino to the repeats and Conserved region II is carboxy to the repeats (Frevert & Crisanti, 1998). It's not completely understood how the parasite crosses the anatomical barrier between liver microcirculation and the hepatocytes. Various possible mechanisms have been described. It is known that both CSP Regions I and II specifically interact with heparin sulfate

proteoglycans (HSPGs) and may interact with the low-density lipoprotein receptor-like protein (LRP) (Sinnis et al., 1996; Frevert & Crisanti, 1998; Nardin & Zavala, 1998).

Amino acid clusters seem to mediate these interactions. Lysines and arginines amino to and in Region I have been identified as glycosaminoglycan (GAG) binding domains. Blocks of basic residues in and carboxy to Region II bind to the sulfated GAG chains of the HSPG family members. The same or similar regions in the molecule may be responsible for the interaction with LRP (Frevert & Crisanti, 1998; Nardin & Zavala, 1998; Sinnis et al., 1996).

The central region of the protein has no defined biological function. The central region is the main target of antibodies developed by individuals naturally exposed to malaria or experimentally immunized with sporozoites. In addition to antibodies, cellular response is important in malaria. T-cell epitopes have been identified in both *Plasmodium falciparum* and *P. vivax* CSP (Nardin & Zavala, 1998).

The CSP gene for *Plasmodium vivax* was cloned and characterized in 1985. The gene is present in the genome as a single copy and shares the characteristics of the same gene of other *Plasmodium* species, a central domain of repetitive amino acid blocks and conserved flanking regions. It was noted that *P. vivax* CSP is more related to that of simian malaria than to that of *P. falciparum* (Arnot et al., 1985; Frevert & Crisanti, 1998).

Rosemberg and collaborators (1989) first described a new form of CSP isolated from patients with parasites microscopically diagnosed as *P.vivax* in Thailand. The parasites in these patients lacked reactivity in an ELISA that used antibodies to the previously reported peptide, GDRA(A/D)GQPA (VK210) for detection. The new variant

presented amino acid blocks differing at 6/9 positions (ANGAGNQPG) and was named VK247. This variant was reported among isolates from Brazil and Papua New Guinea, where they were present in very different prevalence profiles, and the protein possessed an extra nonapeptide block when compared to the original Thai isolate (Qari et al., 1991 and 1992).

In 1990, a variation from the VK210 type was reported from Thailand. In this case deletions of an entire codon from various repeat blocks were presented as evidence that mutations are more likely to occur in the repeat region as a result of errors during DNA replication (Arnot et al., 1990).

At a study site in Papua New Guinea a new form of malaria was identified. The parasite microscopically resembled *P. vivax* but the CSP was identical to that of *Plasmodium simiovale*. Serological cross-reactivity and 18S ribosomal DNA analysis was used to characterize a new human malaria parasite, *Plasmodium vivax*-like. Peptides of this new parasite reacted with sera from Brazil and Papua New Guinea (Qari et al., 1993a). *Plasmodium vivax*-like was detected and sequenced in additional samples from Brazil and Papua New Guinea and in isolates from Indonesia and Madagascar. These data demonstrated the widespread distribution of this parasite (Qari et al., 1993b).

1.5.3.3 The Merozoite Surface Protein (MSP) gene:

The Merozoite Surface Proteins are a family of proteins covering the surface of the merozoite, the parasite stage that invades erythrocytes. Eight members of the MSP family have been identified for *P. vivax*: PvMSP-1, PvMSP-185, PvMSP-3α, PvMSP-3β, PvMSP-3γ, PvMSP-4, PvMSP-5, PvMSP-9 (del Portillo et al., 1991; Barnwell et al.,

1999; Galinski et al., 1999; Black et al., 2002; Vargas-Serrato et al., 2002). A single copy gene encodes the MSP-1 of *Plasmodium vivax* and genetic variation is described in the literature (Del Portillo et al., 1991; Gibson et al., 1992).

Plasmodium falciparum MSP-1_{19} is essential for red blood cell invasion (Blackman et al., 1990). Peptides of the MSP-1 of *P. vivax* have high erythrocyte binding activity, and reticulocyte-binding activity has been demonstrated (Rodríguez et al., 2002). Fragments of the PvMSP-1 have been cloned and expressed to demonstrate their immunological activity (Espinosa et al., 2003). Six T-cell epitopes of PvMSP-1 were identified and characterized. The epitopes bound promiscuously to four different HLA-DRB1* alleles (Caro-Aguilar et al., 2002). Various levels of immune response were observed in studies using MSP-1 as antigen in both artificial (Holder & Freeman, 1981; Perrin et al., 1984; Kumar et al., 1995; Lakshman et al., 1998; Collins et al., 1999; Oliveira et al., 1999) and natural exposures (Mertens et al., 1993; Levitus et al., 1994; Soares et al., 1999a, 1999b).

Comparison between MSP-1 sequences from different malaria species demonstrated the presence of conserved, semi-conserved and variable blocks (Del Portillo et al., 1991; Cooper, 1993; Gibson et al., 1992; Putaporntip et al., 2002). Alignment of various isolates revealed potential recombination sites within and between variable blocks suggesting that allelic variation is generated by intragenic recombination events (Putaporntip et al., 2002). The two monkey-adapted strains, Belém and Salvador, present an overall identity of 81%. The main difference between them is the absence in the Salvador Strain of a 23 glutamine repeat sequence (Gibson et al., 1992).

In genotype studies based on interspecies conserved blocks 5 and 6 of *P. vivax* MSP-1 gene, different types have been described with high levels of heterogeneity between parasite populations (Del Portillo et al., 1991; Gibson et al., 1992; Porto et al., 1992; Premawansa et al., 1993; Cheng et al., 1993; Mancilla et al., 1994; Kolakovich et al., 1996; Putaporntip et al., 1997). The three *P. vivax* MSP-1 types reported are: Belém (Del Portillo et al., 1991), Salvador I (Gibson et al., 1992) and Recombinant (Premawansa et al., 1993; Kolakovich et al., 1996; Putaporntip et al., 1997; Lim et al., 2000; Maestre et al., 2004). These types have been reported in various geographic regions of the world, including Brazil. The diversity of *P. vivax* MSP-1 was used as a genetic marker to determine reintroduction of malaria in areas previously free of transmission, e.g. Italy (Severini et al., 2002) and Uzbekistan (Severini et al., 2004). It was also applied to distinguish recrudescence from reinfection in the assessment of drug sensitivity (Kirchgatter & Del Portillo, 1998; Taylor et al., 2000; Cattamanchi et al., 2003).

2. Methods:

2.1 Review of Epidemiological Data from Manaus:

A manual review of epidemiological data from Manaus was performed using available sources. At the FMT-Am, logbooks are kept of records of all blood smears read in the malaria laboratory. There are separate books for first-time patients, those that arrive in the malaria clinic with a suspected infection, and two follow-up books for those with a positive diagnosis treated as outpatients or admitted to the hospital. In addition, separate books are kept to record specific results used for epidemiological or research projects. The foundation publishes a report every three months. We used these reports as references for the total number of cases diagnosed and total number of admissions to the hospital.

The Brazilian Ministry of Health has an active program to monitor and control malaria in the national territory. It is mandatory for all municipalities and states to report active transmission and numbers of cases diagnosed in their jurisdiction. This information can be accessed from the web pages kept by the different government agencies: Database in Health from the Ministry of Health (MS-DATASUS: http://www.datasus.gov.br); National Foundation of Health (FUNASA: http://www.funasa.gov.br); Secretary of Surveillance in Health (SVS: http://dtr2001.saude.gov.br/svs/). We accessed these data for our study. All data collected were tabulated using Microsoft® Excel, and analyzed using descriptive statistics and graphical charts. We calculated rates, percentages and proportions of malaria and admissions due to malaria from 1980 to 2003.

2.2 Retrospective Cohort study – Overview:

2.2.1 Sample Size and Power Calculation:

To calculate the sample size for this study, we hypothesized that the increased number of admissions for treatment in the hospital observed in the FMT-Am, between 1989 and 2003, reflected an increase in patients presenting in a more debilitated state at time of diagnosis and, consequently, more severely ill than those treated in the outpatient clinic, using the Alecrim (2000) hospital admission criteria. We also hypothesized that this increase in severe disease was linked to a genetic variant that would be present in the admitted group and not in the outpatient group.

Samples used were those collected in the Alecrim cohort study (Alecrim, 2000). To find a difference in genetic profile of approximately 50% between cases and controls with a power of 80%, a total of 30 samples would be required; 10 from patients admitted to the hospital (cases) and 20 from patients treated in the malaria outpatient clinic (controls). Calculations were made using nQuery® 4.0 (Elastoff, 2000).

2.2.2 Samples from The Alecrim Cohort:

Alecrim (2000) performed a descriptive study of patients who spontaneously sought the FMT-Am for diagnosis and treatment of *Plasmodium vivax* malaria during the period from June 1997 to July 1999. The sample size for that cohort was based on 6000 malaria cases diagnosed at the foundation in 1996.

Outpatients received an identification code, corresponding to the file number in the database, and the admission number in the medical records identified patients

admitted to the hospital. During follow-up and course of treatment, some of the patients in both the outpatient clinic and hospital presented with reappearance of parasites in the blood smear.

We randomly selected patients from the admitted and outpatient clinic groups using SPSS. Two subsets were selected from the outpatient clinic: patients who did not present a second time-point infection, “No relapse group”; and patients presenting with a second time-point infection, “Relapse group”. In the relapse group, test sample numbers were assigned the suffix “A” to identify the blood sample collected at the time of initial diagnosis, and “B” to identify the sample collected when parasites were detected in the blood smear for the second time. Patients treated in the hospital, the “Admitted group”, were identified by a letter (A to K). In this group, the patient identified as “K” also had a second episode of parasitemia. The samples corresponding to the first and second infection were identified as KA and KB.

2.2.3 Statistical Analysis:

Patient data were analyzed using SPSS version 12.0.1. Means were compared using Kruskall-Wallis and one-way ANOVA. The relationship between variables was determined by correlation coefficient test. Cross-tabulation was tested using X^2-test.

2. 3 Historical Samples:

Blood smears collected in the Brazilian Amazon Region during the 1970s were kept by Dr. Donald Roberts, USUHS. These samples were used in an effort to determine the gene sequences profile of *P. vivax* present in the region during that period. Slides were reviewed to determine the presence of malaria parasites and photographed. DNA was extracted and genes amplified by PCR and sequences as described below.

2.4 DNA Extraction Protocols and Precautions Used:

General precautions to avoid contamination of samples:

To avoid cross contamination between samples and contamination of DNA stocks with PCR products, DNA extraction was performed in a separate room from the one in which PCR amplification was performed. A designated microcentrifuge was used for DNA extraction only. PCR-amplified product was never handled in the DNA extraction area. Sterile precautions were used for handling of all samples. Work areas were cleaned with bleach between uses. The hood used for PCR setup was treated with UV light between uses.

DNA extract from samples on filter paper (dried blood spots) were stored in 30-50μL aliquots to avoid contamination of the entire stock because of multiple entries. Each sample aliquot was stored in a separate freezer box to be used during PCR for each gene, CSP, MSP-1 and SSUrRNA.

The final volume of DNA extract from samples in blood smears was not enough to divided into multiple aliquots. Historical samples were handled after completion of the handling of the cohort samples from Manaus.

Extraction protocols:

Samples transported as dried blood spots in filter paper:

DNA was extracted using the QIAamp® DNA mini kit from QIAgen (QIAgen Inc., Valencia-CA). The "Dried Blood Spot protocol" was followed as described in the kit instructions:

1. Cut 3-6 pieces (each piece approximately $3mm^2$ cut with fresh razor blades) from the dried blood spot and put into a microcentrifuge tube. Add 180μL of Buffer ATL.

2. Incubate for 85°C for 10 minutes. Briefly centrifuge to remove drops from the lid.

3. Add 20μL of Proteinase K stock solution, mix by vortexing, and incubate at 65°C for 1 hour. Briefly centrifuge to remove drops from the lid.

4. Add 200μL of Buffer AL to the sample, mix by vortexing, and incubate at 70°C for 10 minutes. Briefly centrifuge.

5. Add 200μL of absolute ethanol to the sample and mix by vortexing. Briefly centrifuge.

6. Apply the mixture to the QIAamp spin column. Close and centrifuge (Eppendorf microcentrifuge 5415D) at 8000 RMP for 1 minute. Place the column in a new collection tube and discard the one containing the filtrate.

7. Add 500μL of Buffer AW1. Close the column and centrifuge for 8000 RPM for 1 minute. Place the column in a new collection tube and discard the one containing the filtrate.

8. Add 500μL of Buffer AW2. Close the column and centrifuge for 13000 RPM for 3 minutes. Discard the filtrate and centrifuge at 13000 RPM for one minute.

9. Place the column in a new 1.5 mL microcentrifuge tube, open the column and add 100-150μL of Buffer AE. Incubate at room temperature for 5 minutes and then centrifuge at 8000 RPM for 1 minute.

Samples were aliquoted and stored at –20^{o}C until use in the PCR reactions.

Samples stored as Blood Smears:

DNA was extracted using the Puregene® DNA Purification system from Gentra (Minneapolis, Mn) following the protocol for "DNA extraction from 50μL of dry blood or blood spot/filter paper" adapted as described.

To adjust the volume of solutions used in the DNA extraction we assumed that blood smears contained approximately 50μL of blood. A fresh, disposable razor blade was used for each smear. Gloves were changed between smears and one paper towel was put under each slide and changed before starting the new one.

a. Removing stains and cleaning slides:

Wash the slides for 2 minutes in Methanol, let air-dry. (Wash the slides moving back and forward in a beaker and then spray methanol on the slides individually).

b. Cell Lysis:

Add 600μL of Cell Lysis Solution (specific formulations not provided to the customer) to a 1.5mL eppendorf tube. Wet the smear with 10-20μL of RBC Lysis solution and scrape off the slide using a razor blade. Add the material to the tube containing the remaining Cell Lysis Solution. Add 3μL of Proteinase K Solution

(20mg/mL) and incubate at 55^{o}C overnight. (If possible, invert the tube occasionally during incubation).

c. RNase treatment:

1. Add 3μL of RNase A Solution (4mg/mL) to the cell lysate.

2. Mix the sample by inverting the tube 25 times and incubate at 37^{o}C for 15 minutes.

d. Protein Precipitation:

1. Cool the samples at 4^{o}C for 5 minutes and then at room temperature for 30 minutes.

2. Add 200μL of Protein Precipitating Solution (specific formulations not provided to the customer) to the cell lysate.

3. Vortex vigorously at high speed for 20 seconds.

4. Put on ice bath for 15 minutes.

5. Centrifuge at 13,000 RMP for 3 minutes.

e. DNA Precipitation:

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5mL microfuge tube containing a mixture of 600μL of isopropanol 100% and 1μL of Gentra Glycogen Solution (20mg/mL).

2. Mix the sample by inverting gently 50 times and then place on ice for 15 minutes.

3. Centrifuge at 13,000 RPM (Eppendorf microcentrifuge 5415D) for 5 minutes, the DNA will be visible as a pellet.

4. Pour off the supernatant and drain the tube briefly on clean absorbent paper. Add 600μL of 70% Ethanol and invert the tube several times to wash the DNA pellet.

5. Centrifuge at 13,000 RPM for 1 minute. Carefully pour off the ethanol.

6. Invert and drain the tube on a clean absorbent paper and allow to air dry for 15 minutes.

f. DNA Hydration:

1. Add 10-20μL of DNA Hydration Solution (10mM tris, 1mM EDTA, pH 7.0-8.0).

2. Rehydrate DNA by incubating sample for 1 hour at 65^{o}C. Tap periodically to help disperse DNA.

3. Store at –20^{o}C until use in PCR reactions.

2.5 Polymerase Chain Reaction (PCR) Protocols and Precautions Used:

General precautions to avoid contamination of PCR reagents:

PCR mixes were prepared in a designated hood where assigned pipettes and tips were stored. Disposable lab coats and shoe covers were used while in preparation of the DNA mix. An area around the PCR hood was delimited as a DNA free zone.

No DNA extract or amplified PCR products were handled in the hood. A mini centrifuge was assigned for the DNA-free area to spin PCR reagents and no samples were allowed there. PCR tubes with mixes were kept in ice until addition of templates and transport to PCR thermocycler.

Primers used:

All primers used in this thesis were synthesized at the USUHS Biomedical Instrumentation Center using the Applied Byosystems 3948 Nucleic Acid Synthesis and Purification System on a 40 nMol scale. OPC Column was used to purify all nucleotides after synthesis.

Reference sequences:

Genomic DNA from *Plasmodium vivax* strains Salvador-1 and Belém were donated by Dr. John Barnwell, CDC, Atlanta, Ga. Plasmid clones from the variants VK210, VK247 and *P.vivax*-like were donated by Dr. Altaf A. Lal, CDC, Atlanta, Ga. Nucleotide sequences from other *P. vivax* sequences were obtained from Gene Bank (http://www.ncbi.nlm.nih.gov/).

2.5.1 PCR for Identification of Malaria Parasites in Blood Samples:

A Nested PCR reaction for amplification of a segment of the 18S SSUrRNA gene type A was used for identification of *Plasmodium* species in DNA samples, as has been described (Figure 4; Snounou et al. 1993a, 1993b). The Snounou procedure was modified for use in this Project. The first reaction used primers homologous to a conserved region common to the genus *Plasmodium*. Aliquots of DNA resulting from these first reactions were then amplified in three independent reactions to synthesize *P. vivax*, *P. falciparum* and *P. malariae* specific DNA. A reaction for *P. ovale* was not used, because this parasite is not present in the Americas.

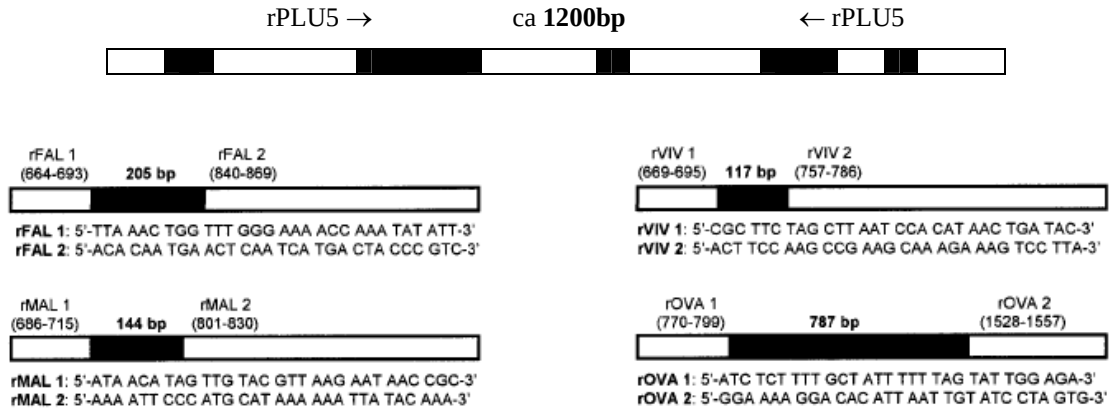


Figure 4: Schematic representation of the 18S SSUrRNA gene and location of primers used for parasite identification according to Snounou et al. (1993).

a. Reagents used:

We used the HotStarTaq® DNA Polymerase Kit, 1000 U and 10mM dNTP mix solution, in the PCR reaction, detailed descriptions of each reagent are included in the annex of this thesis.

We used primers described by Snounou et al. (1993). To amplify the genus *Plasmodium* 18S SSUrRNA gene we used, PLU5: 5'CCT GTT GTT GCC TTA AAC TTC 3' and PLU6: 5'TTA AAA TTG TTG CAG TTA AAA CG 3'. A second reaction was used to amplify SSUrRNA gene of each of the *Plasmodium* species. For the *P. falciparum* reaction we used FAL1: 5'TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT 3' and FAL2: 5'ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC 3'. For the *P. vivax* reaction we used VIV1: 5'CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC 3' and VIV2: 5'ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA 3', for the

P. malariae we used MAL1 5'ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC 3' and MAL2 5'AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA 3'

b. Expected size of the amplicons according to Snounou et al. (1993):

The *Plasmodium* reaction amplifies a 1.2 Kb fragment, the *P. vivax* reaction amplifies a 120 bp fragment, the *P. falciparum* reaction amplifies a 205 bp fragment and the *P. malariae* reaction amplifies a 144 bp fragment.

c. Reaction conditions and cycle used:

The *Plasmodium* sp. reaction was performed using 1-5μL of template DNA in a total volume of 50 μL containing 1x PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 1x Q Solution (formulation not provided to the customer), 0.125 mM dNTP mix, 13 pMol of each primer (PLU5 and PLU6) and 2.5U of HotStarTaq DNA Polymerase.

The species-specific reactions were carried out as 3 different reactions (*Plasmodium vivax*, *P. falciparum* and *P. malariae*) using 1-5μL of the PCR product from the first reaction in a total volume of 20μL containing 1x PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 1x Q Solution, 0.125 mM dNTP mix, 5 pMol of each primer (VIV1 and VIV2 for *P. vivax*; FAL1 and FAL2 for *P. falciparum*; MAL1 and MAL2 for *P. malariae*) and 2.5U of HotStarTaq DNA Polymerase.

All reactions included an initial cycle of 95^oC for 15 minutes, followed by 30 cycles of 94^oC for 1 minute, 55^oC for 2 minutes and 72^oC for 1 minute. For amplification of DNA from blood smear the total number of cycles was increased to 40. A final extension cycle was performed at 72^oC for 10 minutes. Samples were then kept at 4^oC

until removed from the thermocycler, 5-8μL of the PCR product aliquots were tested by agarose gel electrophoresis to determine the presence and size of amplicons.

2.5.2 PCR for Amplification of Target Region in the 18S Small Subunit Ribosomal RNA (18S SSUrRNA) Type A gene:

A nested PCR reaction was designed to amplify a target area between variable regions 7 and 8 of the 18S SSUrRNA Type A gene from *P. vivax* (Figure 5). This is the region described as containing a mutation in a sample from a patient presenting relapsing disease diagnosed and treated at the FMT-Am. The mutation was considered to represent a possible marker of a pathogenicity variant of *P. vivax* (Alecrim, 1999).

a. Reagents Used:

We used the HotStarTaq® DNA Polymerase Kit, 1000 U and 10mM dNTP mix solution in the PCR reaction, details for each reagent are included in the annex of this thesis.

We designed four primers to be used in a nested PCR reaction, for the first reaction we used the primers pairs VAR1 - 5' CTT GGA TGG TGA TGC ATG GCC 3' and VAR2 - 5' ATC TTT CAA TCG GTA GGA GCG AC 3'. For the second reaction we used VAR3 - 5' CGT GAA TAT GAT TTG TCT GG 3' and VAR4 - 5' CAA TAA TCT ATC CCC ATC ACG 3'.

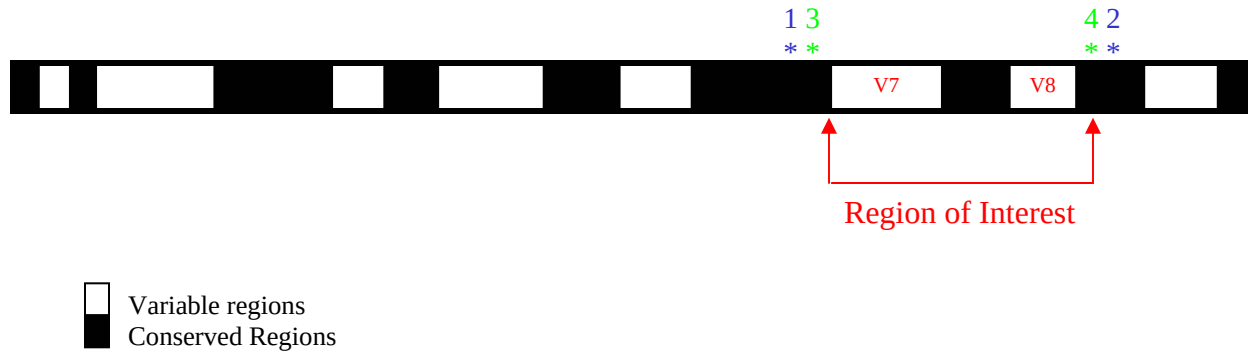


Figure 5: Schematic representation of the 18S SSUrRNA Type A gene and location of the region of interest for phylogenetic analysis (Adapted from Li et al., 1995).

b. Expected size of the amplicons based on sequences available at Gene Bank NCBI:

The size of the amplicons were estimated based on sequences the with access numbers PVU03080 and PVU03079, available at Gene Bank. Using primer pairs VAR1/2 a 575 bp and 639 bp fragment is expected. For reaction using primer pairs VAR3/4 a product of 478 bp and 414 bp is expected.

c. Reaction conditions and cycle used:

From samples with confirmed infection by *P. vivax*, 1-5µL (10µL for some isolated from blood smears) of DNA extract was amplified in a 50µL reaction containing 1x PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 1x Q Solution, 0.2 mM dNTP mix, 50 pMol of each primer (VAR1 and VAR2) and 2.5U of HotStarTaq DNA Polymerase. PCR product from the first reaction (1-5µL) was used in a second amplification using the primers VAR3 and VAR4 in conditions identical to the first reaction.

All reactions were submitted to an initial cycle of 95°C for 15 minutes, followed by 30 cycles of 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute, a final extension at 72°C for 10 minutes, and a final hold of 4°C until removed from the thermocycler. In the event of little or no amplification, the number of cycles was increased to 40 in both reactions. The PCR product (5-8μL) was used for agarose gel analysis to determine the presence and size of amplicons.

2.5.3 PCR for Amplification of Target Region in the Circumsporozoite Surface Protein (CSP) gene and Restriction Fragment Length Polymorphism Analysis (RFLP):

Variation in the *Plasmodium vivax* CSP gene was recognized relatively recently (Arnot et al., 1985; Rosemberg et al., 1989; Arnot et al., 1990). Three variants are presently recognized: VK210, VK247, and *vivax-like* (see above). Using sequences available in the Gene Bank (http://www.ncbi.com), primers were designed and tested for application in PCR using plasmid DNA clones as templates.

Conserved regions of the gene coding for CSP allowed the design of internal and external primers capable of amplifying all the three described variants of *P. vivax*, as illustrated in Figure 6. These primers were used in nested and semi-nested PCR reactions to amplify parasite DNA from the samples included in this study. The first amplification was performed using either pair of primers, P1 and P2 or M-1 and M2. The second amplification involved a nested and/or a semi-nested PCR reaction using the primer pairs N1 and N2 or N1 and P2 respectively. An additional primer, CSP-A2, described by Lim et al. (2001), was also used for sequence reactions, as follows:

a. Reagents used:

We used the GeneAmp® XL PCR Kit and HotStarTaq® DNA Polymerase Kit and 10mM dNTP mix solution in the PCR reaction, details for each reagent is included in the annex of this thesis.

We designed primers used for the PCR reaction. The first reaction used primer pairs P-1: 5' AAT GTA GAC GCC AGT TCA CT 3' and P-2: 5' GCT AGG ACT AAC AAT ATG AC 3', or M-1: AAT GTA GAC GCC AGT TCA CTT 3' and M-2: 5' GCT AGG ACT AAC AAT ATG ACT AG 3'. For the second reactions we used primer pairs N-1: 5' AGG CAG AGG ACT TGG TGA GA 3' and N-2: 5' TCA TTT GTG GCA TTC GCA CCT TC 3' or N1 and P2. The primer described by Lim et al. (2001), CSP-A2: 5' TCT AGA GAA AAT AAG CTG AAA CAA CCA GGA 3' was used for sequencing reactions.

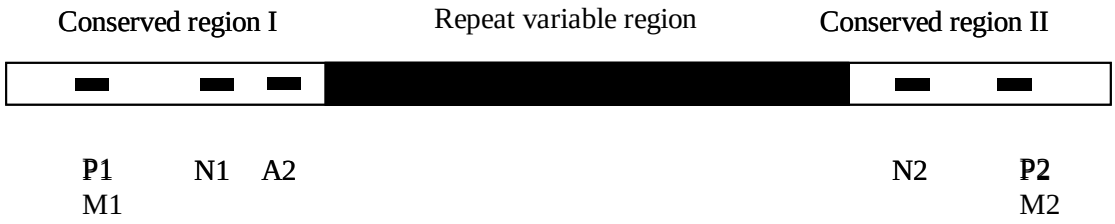


Figure 6: Schematic representation of the CSP gene and location of primers used for amplification of target region and/or sequencing.

b. Expected size of the amplicons based on sequences available at Gene Bank NCBI:

The sizes of the amplicons were estimated based on sequences of *P. vivax* CSP variants available at Gene Bank. Using primer pairs, P1/P2 or M1/M2 we expected the following results: for *P. vivax* VK210, a 979 bp fragment based on the sequence with access number L05068 and 955 bp fragment based on the sequence with access number M11926; for *P. vivax* VK247, a 1009 bp fragment based on the sequence with access number L05069 and 1006 bp for the sequence with access number M69059; for *vivax*-like, a 1000 bp fragment based on the sequence with access number L13724

Using the primer pairs N1/N2 in the second reaction, the following results were expected: for *P. vivax* VK210, a 734 bp fragment based on the sequence with access number L05068 and a 710 bp based on the sequence with access number M11926; for *P. vivax* VK247, a 764 bp fragment based on the sequence with access number L05069 and a 761 bp fragment based on the sequence with access number M69059; for *P. vivax*-like a 755 bp fragment based on the sequence with access number L13724.

Using the primer pairs N1/P2 in the second reaction, the following results were expected: for *P. vivax* VK210, a 926 bp fragment based on the sequence with access number L05068 and a 902 bp fragment based on the sequence with access number M11926; for *P. vivax* VK247, a 956 bp fragment based on the sequence with access number L05069 and 953 bp fragment based on the sequence with access number M69059; for *P. vivax*-like a 947 bp fragment based on the sequence with access number L13724.

c. Polymerase Chain Reaction conditions and cycle used:

P1/P2 – M1/M2 Reaction:

We used the GeneAmp® XL PCR Kit, which contains a proof reading enzyme, as follows: 1-3 μL of DNA extract was added to a mix of 1x XL Buffer II (Tricine, Potassium Acetate, Glycerol, DMSO), 0.9mM $Mg(OAc)_2$, 0.4mM of dNTP mix, 50pMol of each primer (P1 and P2 or M1 and M2) and DEPC water (water treated with diethyl pyrocarbonate - C6H10O5, 0.03%) to complete 50μL. The samples were heated for 5 minutes at 95°C prior to addition of 1U of rT*Th* Polymerase. Amplification occurred in 30 cycles of 94°C for 1 minute and 62°C for 8 minutes, followed by a final extension at 72°C for 10 minutes then a holding step of 4°C until products were removed from the machine.

N1/N2 Reaction:

We used the GeneAmp® XL PCR Kit, which contains a proof reading enzyme, as follows: 1-3 μL of the first PCR reaction (P1/P2 reaction) was added to a mix of 1x XL Buffer II (Tricine, Potassium Acetate, Glycerol, DMSO), 0.8mM $Mg(OAc)_2$, 0.4mM of dNTP mix, 50pMol of each primer (N1 and N2) and DEPC water to complete 50μL. The samples were heated for 5 minutes at 95°C prior to addition of 1U of rT*Th* polymerase. Amplification occurred in 30 cycles of 94°C for 1 minute and 65°C for 8 minutes, followed by a final extension at 72°C for 10 minutes then a holding step of 4°C until products were removed from the machine.

N1/P2 Reaction:

We used the HotStarTaq® DNA Polymerase Kit, which contains a high fidelity polymerase, as follows: 1-5 μL of PCR product from the P1/P2 or M1/M2 reactions was added to a mix of 1x HotStarTaq® PCR Buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 1x Q-Solution, 0.4 mM of dNTP mix, 50pMol of each primer (PV1/PV2), 2.5U of HotStarTaq® DNA Polymerase and DEPC water to complete 50μL. PCR tubes were submitted to a cycle of 95°C for 15 minutes; 39 cycles of 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, followed by a final cycle of 95°C for 1 minute, 60°C for 1 minute and 72°C for 10 minutes, then a holding step of 4°C until products were removed from the machine.

d. Restriction Fragment Length Polymorphism (RFLP) analysis:

Using the MapDraw 3.08b program in the DNASTAR software package (DNASTAR, Inc.), we compared restriction maps from several sequences of *P. vivax* variants VK210, VK247 and *Plasmodium vivax*-like available in Gene Bank. Based on these analyses we chose restriction enzymes predicted to selectively digest each of the variants. In each case the restriction enzyme recognition sites were located in the repeat region of the amplicons.

For the *P. vivax* variant VK210 we chose the restriction enzyme PVU II, predicted to digest the repeat block GDRADGQPA. For the VK247 variant we chose Bcl I, predicted to digest the repeat block ANGAGNQPG. For the *P. vivax*-like variant we chose the BxtX I, predicted to digest the repeat block APGANQEGGAA.

PVU II recognizes the site 5’ CAG ˇ CTG 3’ in the *P. vivax* VK210 genotype, and is the same enzyme selected by Kho et al. (1999) to study the variants in Korea. Bcl I

recognizes the site 5' T ˇ GATCA 3' in the *P. vivax* VK247 genotype. BxtX I recognizes the site 5' CCANNNNNˇNTGG 3' *P. vivax*-like genotype.

In each case, digestion was carried out in a 1.5 mL microcentrifuge tube, to which was added 10μL of PCR product, 5 Units of restriction enzyme, 1x NE Buffer 2 (50mM NaCl, 10mM Tris HCl, 1mM dithiothreitol) or 3(100mM NaCl, 50mM Tris-HCl, 10mM $MgCl_2$, 1mM dithiothreitol), as appropriate, and DEPC water to a total volume of 20μL. The digestions were carried out at 37^o, 50^o or 55^oC for 1 hour, for PVU II, Bcl I, or BstX I, respectively.

2.5.4 PCR for Amplification of the Target Region in the Merozoite Surface Protein-1 (MSP-1) gene:

The region of the MSP-1 gene between the Interspecies Conserved Blocks (ICB) 5-6 has been studied by different authors to determine diversity of *Plasmodioum vivax* parasites (Figure 7, Porto et al., 1992; Premawansa et al., 1993; Mancilla et al., 1994; Putaporntip et al., 1997; Lim et al., 2000) and to compare initial infection with relapse in different regions of the globe (Kirchgatter & Del Portillo, 1998; Taylor et al., 2000; Cattamanchi et al., 2003).

We chose this region as a target for our studies. A set of primers previously described (Putaporntip et al., 1997) was used during the first round amplification, and an additional set of primers designed for this project was used in a second round, nested PCR reaction and for sequencing of PCR products.

a. Reagents used:

We used the HotStarTaq® DNA Polymerase Kit and 10mM dNTP mix solution in the PCR reaction, details for each reagent is included in the annex of this thesis.

For the first PCR reaction, we used the primer pairs PV1: 5' GAA ATT TAT GAT CTA GCC CAG GAA ATC CG 3' and PV2: 5' GTT TCC AGG AAA GCC TTA ATC TTC TTG TTC 3' described previously (Putaporntip et al., 1997). For the second reaction we designed the primer pairs 5F1: 5' GAC GAC CTG TAC GTT CCA 3' and 6R1: 5' TCC TTC TGG TAC AGC TCA 3'.

Sequencing reactions were performed using primer pairs 5F1, 6R1 and SQ1: 5' GGA AAT TGA CAA GTT GAA GG 3' and SQ2: 5' GTG CTT GTG ACA TGC GTA AG 3' described by Putaporntip et al. (1997).

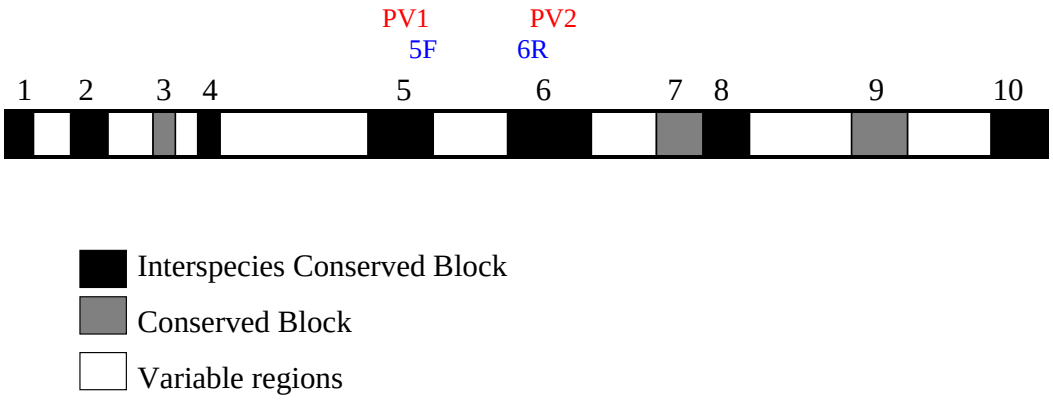


Figure 7: Schematic representation of the MSP-1 gene (Adapted from Del Portillo et al., 1991 and Gutierrez et al., 2000)

b. Expected size of the amplicons:

The sizes of the amplicons were estimated based on *P. vivax* MSP-1 sequences available at Gene Bank. Using primer pairs PV1/2 we expected a 949 bp fragment based on the sequence with access number AF435594, and a 985 bp fragment based on the sequence with access number M75674. For the reaction using primer pairs 5F/6R reaction we expected a 715 bp fragment based on the sequence with access number AF435594, and a 764 bp fragment based on the sequence with access number M75674.

c. Reaction conditions and cycle used:

1-5 μL of DNA extract was added to a mix of 1x PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 1x Q-Solution, 0.2 mM of dNTP mix, 50pMol of each primer (PV1/PV2), 2.5U of HotStarTaq® DNA Polymerase and DEPC water to complete 50μL. PCR tubes were submitted to a cycle of 95°C for 15 minutes, 39 cycles of 95°C for 1 minute, 55°C for 2 minute and 72°C for 1 minute, then a final cycle of 95°C for 1 minute, 55°C for 1 minute and 72°C for 10 minutes, followed by a holding step of 4°C until products were removed from the machine. The following amplification using 5F/6R primers was conducted using 1-5 μL of the PCR product in conditions identical to the first reaction. PCR product (5-8μL) was tested by agarose gel electrophoresis to determine the presence of amplicons in the expected size.

2.5.5 Processing of PCR Products:

After amplification and confirmation of the expected size of amplicons by agarose gel, the PCR products were submitted to purification and direct sequencing. If direct sequencing was not successful, the PCR product was cloned and sequences of the clones were determined.

All PCR products were purified using the QIAquick PCR Purification Kit following the directions provided with the kit. Samples were eluted with either 30 or 50 μL of elution buffer, based on intensity of the band in the agarose gel. All sequencing was performed in the USUHS BIC using Applied Biosystems 3100 and big dye versions 2, 3.0 and 3.1 chemistry. For direct sequencing of PCR products, 5-10 μL of each purified PCR product was mixed with 4 μL of Big Dye mix, sequencing primer (200-400 pMol/ reaction) and water to complete 20 μL in a PCR tube on ice. Sequencing reactions used a basic cycle of 25 rounds at 95^{o}C for 10 seconds, annealing temperature (AT)^{o}C for 4 minutes and 10 seconds, and a final hold at 4^{o}C.

For the 18S SSUrRNA Type A gene, sequencing reactions were performed using primers Var3 and Var4 with AT of 60^{o}C. CSP gene PCR products were sequenced with the primers CSP-A2, N1, N2 and P2 and AT of 60^{o}C. MSP-1 gene PCR products were sequenced with SQ-1, SQ-2, 5F1 and 6R1 and AT of 55^{o}C. After sequencing reactions were completed, products were separated from unreacted terminator dye using Performa® DTR Gel Filtration Cartridges (Edge Biosystems). PCR tubes were spun down to remove any drops from the lid. Performa® column was first spun at 4000 RPM for 1 minute. Reaction products were then applied to the dried column and centrifuged at 3000 RMP

(Eppendorf microcentrifuge 5415D) for 2 minutes. Product was then submitted for sequence determination using the sequencing machine.

For cloning PCR products, two cloning strategies were used in this project. The PCR-Script^{TM}Amp Cloning Kit (Stratagene) and the Topo-TA Cloning Kit (Invitrogen) were utilized, following the specifications provided with each of the kits. Once products were isolated from individual clones, they were sequenced as described in direct sequencing of PCR products.

The sequence results were provided as a printed histogram and text file. The text files were edited using EditSeq 3.88 (DNASTAR inc.). Forward and reverse sequences were assembled using SeqMan 3.03 (DNASTAR inc.). Edited sequences were aligned using Megalign 3.06b (DNASTAR inc.) and Clustal X 1.83 (Thompson et al. 1997). Files were saved in the following formats: *.meg, *.msf, *.aln, *.nxs, and *.fasta for further analyses.

2.5.6 Sequence Analysis and Phylogeny:

Nucleotide composition:

MEGA 2.1 (Kumar et al., 2001) was used to determine nucleotide frequencies and nucleotide pair frequencies.

Tree search and Bootstrap analysis:

PAUP 4.0b10 (Swofford, 2002) and MEGA 2.1 (Kumar et al. 2001) were used to build phylogenetic trees using Distance and Parsimony approaches. Bootstrap was used to test the reliability of the groups found. The scripts (Annex) used for PAUP were based on those posted on the Internet (Bielawski, 2003; Salemi & de Oliveira, 2003).

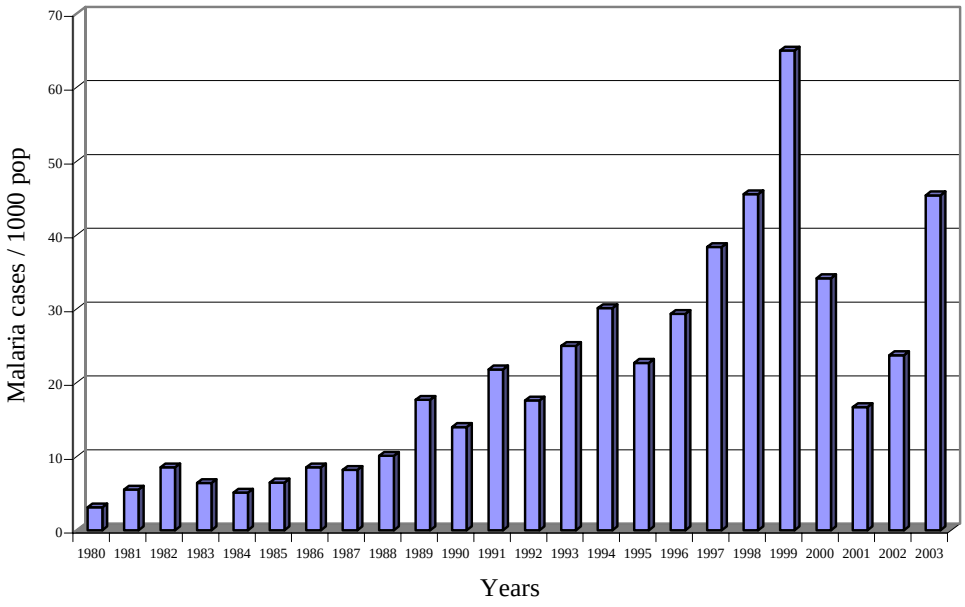
3. Results:

3.1 Epidemiology of Malaria in the Amazonas State and City of Manaus:

Data from the Brazilian Ministry of Health shows that the northern region of the country is responsible for 98.81% of the total malaria cases in Brazil. The Amazonas state contributes 34.4% of this total, followed by the States of Pará (29.6%) and Rondônia (24.2%) (SVS, 2004).

Despite different control strategies applied by the government, the rate of malaria increased between 1980 and 2003. The rate of malaria per 1000 population (Figure 8) in the Amazonas State increased from 3.10 in 1980 to 45.37 in 2003, reaching a maximum of 64.98 in 1999 (SVS, FUNASA, DATASUS, 2004, PAHO, 2003).

Figure 8: Malaria rates in Amazonas State, Brazil, from 1980 to 2003.



Data source: MS: SVS, FUNASA

Rate: (Malaria all forms/Population of Amazonas) x 1000

From 1980 through 1988, the rate of malaria cases per 1000 population was below 10. Since 1988, the rate has increased irregularly, reaching the rate of 64.98 cases /1,000 population in 1999. In 2001, a significant reduction to 16.86/1,000 was registered, but cases started rising again, reaching 23.70/1,000 in 2002 and 45.37/1,000 in 2003.

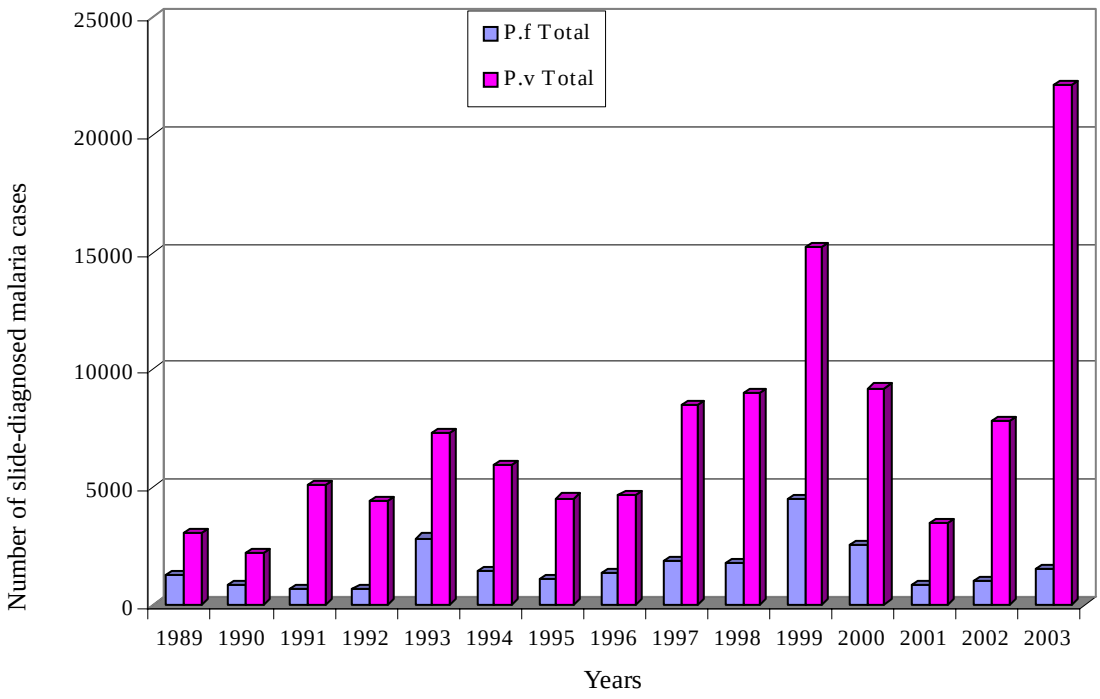
The proportion of total cases in Amazonas State that were diagnosed and treated at the FMT-Am has been relatively constant, varying in proportion to the fluctuations that occurred through out the State (Table 1). Variations in the numbers of cases diagnosed annually of both *Plasmodium falciparum* and *P. vivax* infections occurred during the period between 1989 and 2003. Peaks occurred in 1993, 1999, and 2003, with surrounding nadirs occurring in 1990, 1995 and 2001 (Figure 9). This type of variation could be attributed to the intensity of mosquito control measures, climatological variation and other factors.

Table 1: Annual number of malaria cases diagnosed in Amazonas State and the Fundação de Medicina Tropical do Amazonas by year, from 1989 to 2003.

Year	Number of cases in Amazonas	Number of cases at FMT-Am	Percent of cases diagnosed in the FMT-Am
1989	34,944	4,347	12.44
1990	28,479	3,037	10.66
1991	45,849	5,765	12.57
1992	37,885	5,083	13.41
1993	55,364	10,157	18.34
1994	68,287	7,469	10.93
1995	52,602	5,765	10.95
1996	70,044	6,206	8.85
1997	94,382	10,483	11.1
1998	114,748	10,854	9.46
1999	167,722	19,967	11.9
2000	96,026	12,266	12.77
2001	48,386	4,315	8.91
2002	70,223	8,871	12.63
2003	137,532	23,744	17.26

Data source: MS: SVS, DATASUS, FMT-Am.

Figure 9: The annual number of malaria cases due to *P. falciparum* and *P. vivax*, by year diagnosed at Fundação de Medicina Tropical do Amazonas, from 1989 to 2003.



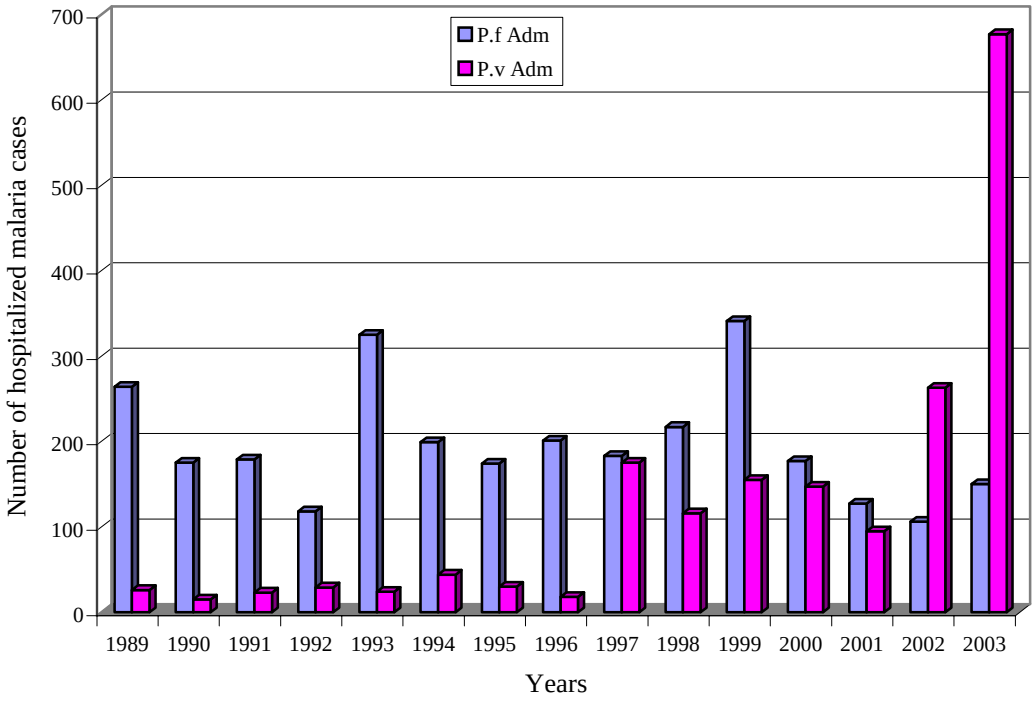
Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am)

Plasmodium vivax was the main cause of malaria diagnosed in the FMT-Am. Of the 4,347 total cases of malaria diagnosed in 1989, *vivax* infections represented 70.0% of the cases (n=3,043) while *falciparum* infections represented 29.0% (n=1262). In 1990, the numbers decreased slightly with 839 cases caused by *P. falciparum* and 2,175 cases due to *P. vivax*. The percent of the total number of malaria cases registered in the FMT-Am (n=3,037) remained about the same, at 27.6% for *P. falciparum* and 71.6% for *P. vivax*. In the years 1991 and 1992, *P. vivax* cases represented 88.0% (n=5,076) and 86.5% (n=4,398) of the malaria cases in the FMT-Am. From 1993 to 1996, there was a reduction in the total number of malaria cases. The percent of the cases due to *P. vivax* was around

70% during this period. In 1997, the total number of malaria cases registered increased from 6,206 to 10,483. *Plasmodium vivax* caused 81.1% of the infections while *P. falciparum* caused 17.8%. From 1998 to 2002, the number of *P. vivax* cases ranged from 9,004 in 1998 to 7,808 in 2002, with a peak of 15,238 in 1999. In 2003, the total number of malaria cases diagnosed in the FMT-Am was 23,744. *P. vivax* caused 22,148 cases and *P. falciparum* caused 1,503 of the cases.

In the FMT-Am, patients diagnosed with malaria, based on clinical and laboratory criteria, were treated as outpatients in the malaria clinic or admitted to the Foundation's hospital. According to the Principal Investigator of the Malaria Laboratory, criteria for admission have not changed much since the mid 1980s. The total number of admissions to the hospital for treatment of malaria is presented in Figure 10. *Plasmodium falciparum* was the main cause of malaria admissions until 1997, when *P. vivax* admissions started to increase. Through 1996, *P. vivax* admissions reached a maximum of 19.7%, since then the admissions irregularly increased until it reached 81.6% (n=677) in 2003. Admissions for *P. vivax* infections exceeded admissions for *P. falciparum* infections in 2002 and 2003.

Figure 10: Total number of admissions at FMT-Am due to malaria, by year and *Plasmodium* species, from 1989 to 2003.



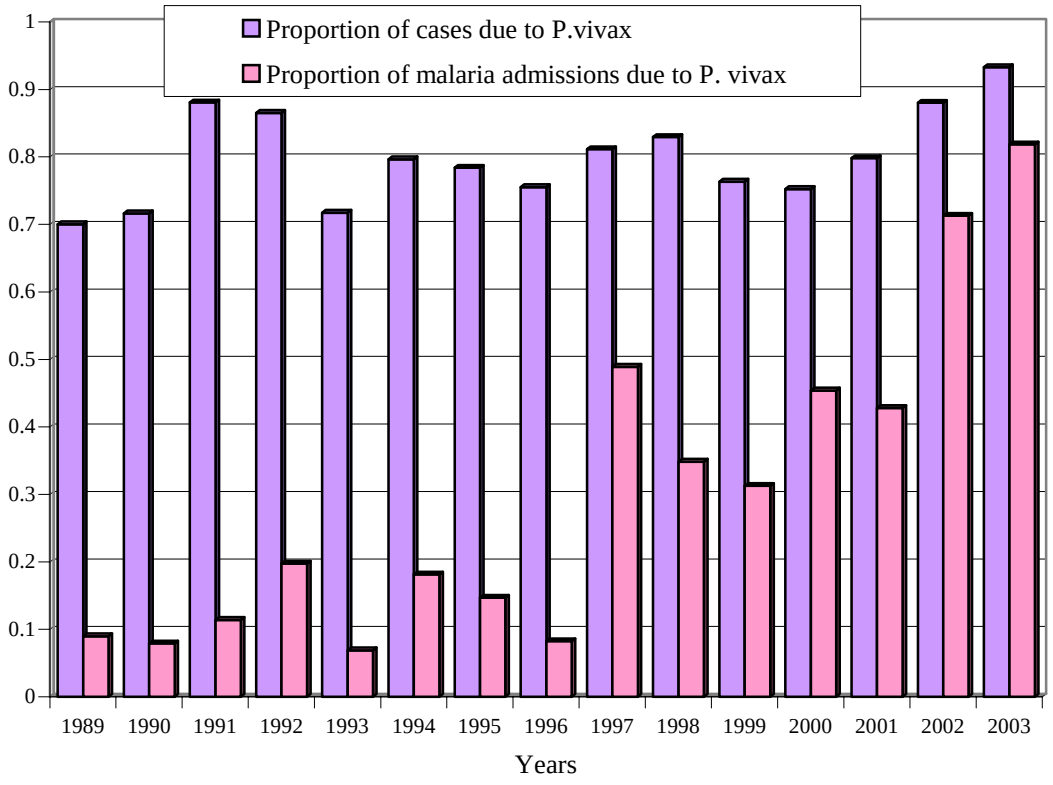
Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am)

As presented previously, the most common malaria infection diagnosed in the FMT-Am was *P. vivax*. We conducted additional analyses to test whether the absolute increase in admissions presented in Figure 10 reflected the increase in total cases of *vivax* malaria (Figure 9).

Figure 11 shows the proportion of cases (number of cases due to *P. vivax* /total malaria cases) and admissions (admissions due to *P. vivax*/total admissions) observed in the past 15 years. From 1989 to 2002, the proportion of cases caused by *P. vivax* ranged from 0.70 to 0.88. In 2003 the proportion of *vivax* cases increased to 0.93. From 1989 to 1996, the proportions of *P. vivax* cases being admitted for treatment over the total

admissions due to malaria were less than 0.1. Since that year, the proportion of *P. vivax* admissions has increased significantly ranging from 0.31 to 0.81.

Figure 11: *Plasmodium vivax*, proportion of cases and admissions from 1989 to 2003. Calculations are the number of cases due to *P.vivax* over total cases of malaria and number of admissions due to *vivax* malaria over the total cases admitted for malaria.

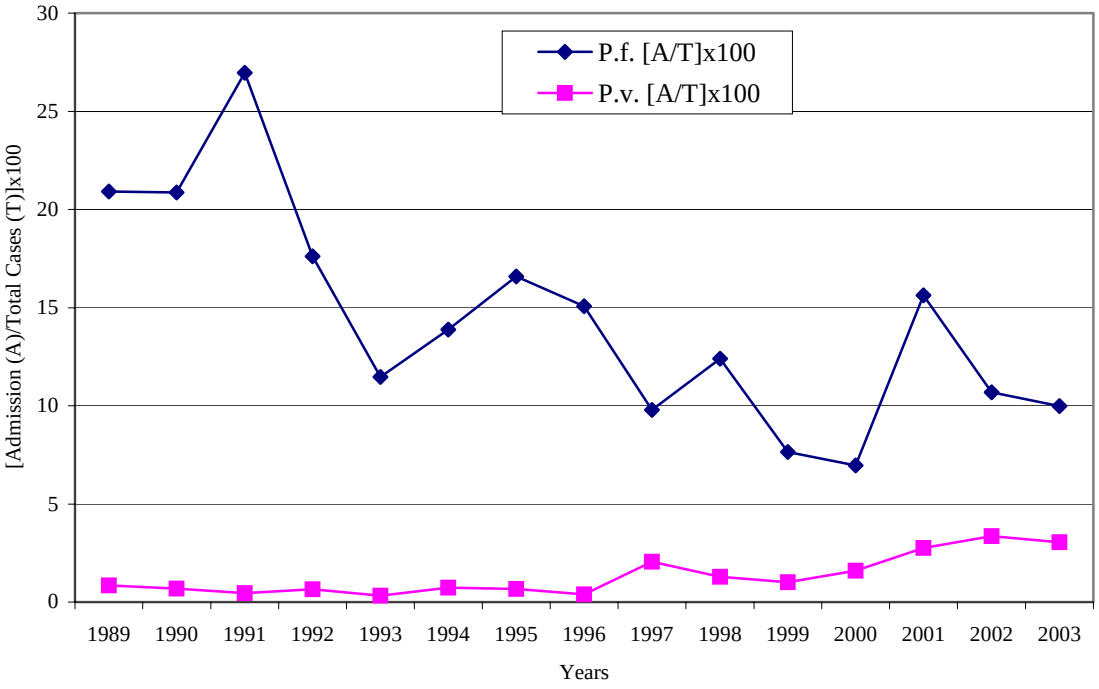


Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am)

Proportion: Cases due to *P. vivax*/Total malaria cases and Admissions due to *P. vivax*/Total Admissions

As depicted in Figure 12, the percent of admissions with *vivax* malaria was low until 1996, when the percent began to increase significantly over the following years. The percent of admissions diagnosed with *P. falciparum* fluctuated irregularly during the time period observed.

Figure 12: Percent admission for each *Plasmodium* species by year, from 1989 to 2003.



Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am)

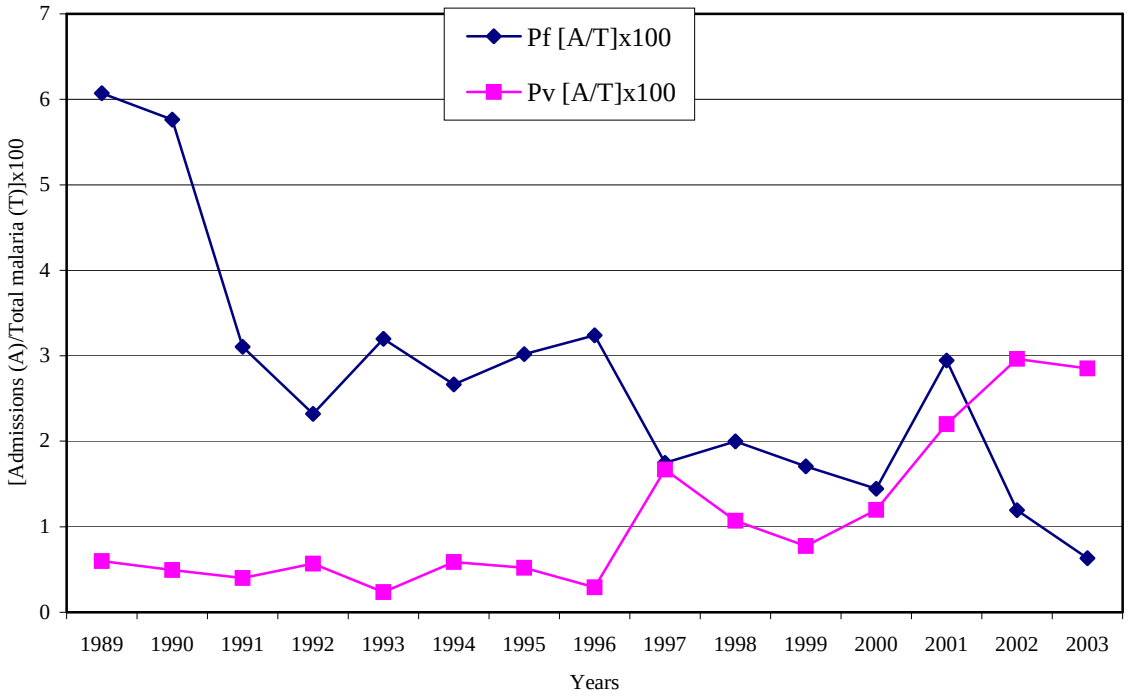
Percent Admissions=(Number of admissions for each *Plasmodium* specie/cases due to the same *Plasmodium* specie)*100

To estimate the risk of being admitted to the hospital for treatment due to illness from a particular parasite species, we calculated the percent of admissions by parasite species and year, using total malaria cases, i.e. caused by all species, as the denominator (Figure 13). Given a malaria diagnosis, hospital admission due to *P. vivax* was less than 0.6% through 1996. In the following years, the proportions increased significantly

ranging from 0.77% to 2.96%. In the time period observed, admissions due to *P. falciparum* decreased.

The data presented here indicate that *P. vivax* malaria had caused unusually frequent, severe disease requiring admission to the hospital for treatment. This change cannot be attributed to a higher number of infections due to *P. vivax* because this parasite has always caused more than 70% of the malaria cases (Figure 11).

Figure 13: Percent admissions over the total malaria cases by year and *Plasmodium* species

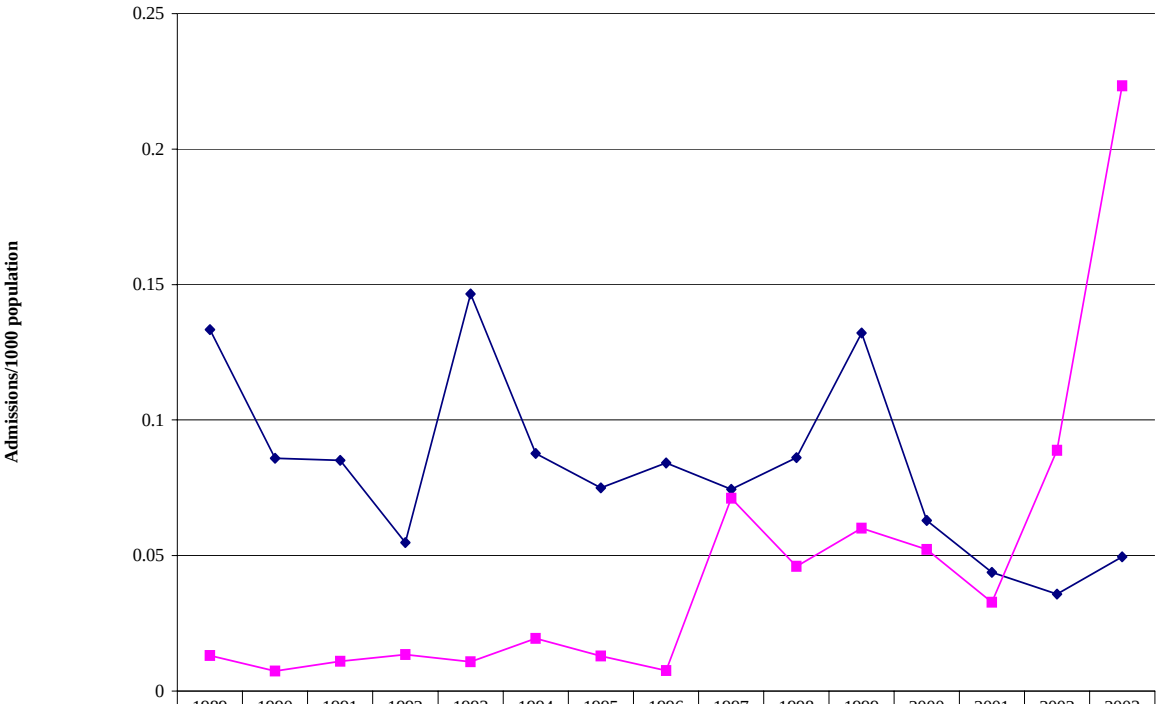


Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am)

Percent admissions=(number of admissions for each *Plasmodium* specie/total cases of malaria)*100

Transfer of severe patients from other institutions for treatment at the FMT-Am could have artificially increased the number of admissions due to *P. vivax*. We investigated this possibility using the state population as the denominator to calculate the rate of admissions. The increase in the rate of *P. vivax* admissions is represented in Figure 14. *Plasmodium falciparum* rates decreased while *P. vivax* rates increased, with rates ranging from 0.007 to 0.22.

Figure 14: Rate of admissions per 1000 population in the Amazonas State, Brazil, by year and *Plasmodium* species, from 1989 to 2003.



	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
P. falciparum	0.13336	0.08591	0.08511	0.05475	0.14656	0.08768	0.07499	0.08413	0.07437	0.08609	0.13213	0.06293	0.04379	0.03579	0.04949
P. vivax	0.01313	0.00736	0.01094	0.01346	0.01082	0.01939	0.01293	0.00753	0.07112	0.04602	0.06006	0.05227	0.03276	0.0888	0.22335

Data Source: Bulletins and logbooks from the Malaria Lab (FMT-Am), IBGE

Rate of Admissions=Number of admissions for each *Plasmodium* specie/1000 Total Population in the Amazonas State

3.2 Phylogeny of *Plasmodium vivax*:

3.2.1 Historical Blood Smears:

First, we analyzed malaria thick smear slides dating from the 1970s on the microscope to observe the presence of malaria parasites (Figure 15). Parasites with morphology consistent with *P. vivax* were identified in all slides. A DNA extraction method was optimized, as described in the Methods section. After several attempts, we obtained PCR products using protocols for amplifying the 18S SSUrRNA Type A and MSP-1 genes (Figure 16).

Direct sequencing of the PCR products was not possible. The PCR product was purified and cloned, according to the instructions of the TOPO cloning kit. Sequences of several clones were obtained, however, they corresponded to the sequences of fungal and bacterial SSUrRNA and other protein genes.

Figure 15: Photograph of historical blood smear, with an arrow pointing to the malaria parasite.

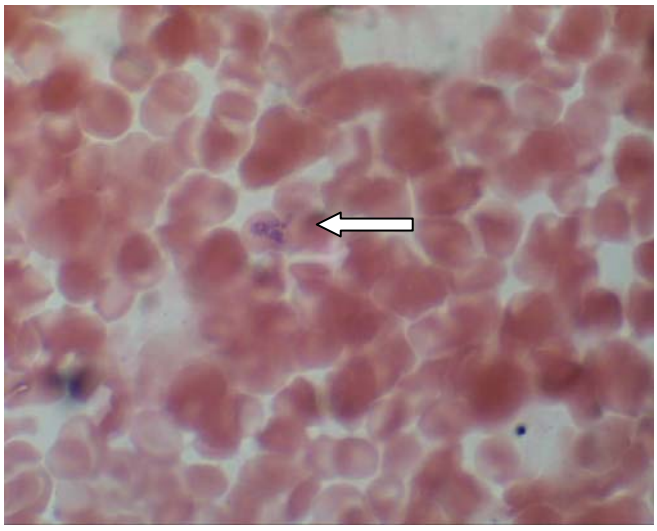
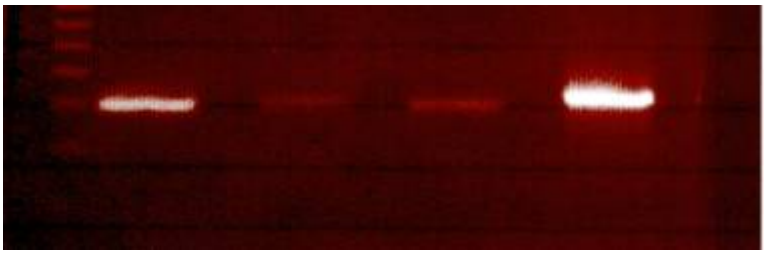


Figure 16: Polymerase chain reaction products from DNA extracted from blood smears collected from patients with malaria in the Amazon Region in the 1970s. A: Nested reaction to amplify the 18S SSUrRNA Type A gene using primers pairs Var1/2 and Var3/4. Template DNA was extracted from blood smears (S1-3) and the positive control (+). B: PCR to amplify the region between interspecies conserved blocks (IBCs) 5 and 6 from the MSP-1 gene using primers pairs PV1/2 and 5F1/6R1. Template DNA was extracted from blood smears S1, S2 and S3, and positive control (+). Electrophoreses were performed on 1.5% agarose gel in Tris acetate-EDTA buffer and stained with ethidium bromide. M: 100 base pairs DNA marker.

A: M S1 S2 S3 +



B: M S1 + +

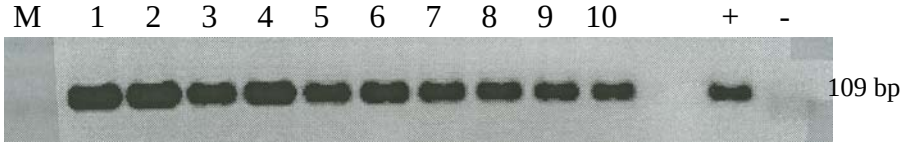
3.2.2 Parasite Identification:

The polymerase chain reaction (PCR) for identification of malaria parasites, as described by Snounou et al. (1993), was modified from its original protocol and applied to the samples included in this project. The test was used to confirm the presence of *P. vivax* and to exclude mixed infections from other malaria parasites endemic in the study region.

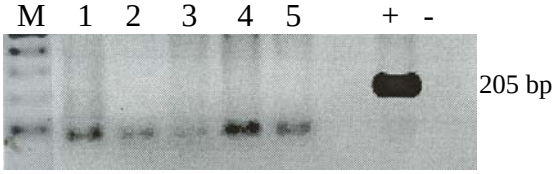
In accordance with the protocol, all samples were submitted to a primary reaction with primers designed to amplify a conserved region of the 18S SSUrRNA Type A gene, a gene present in *Plasmodium* parasites affecting humans. The PCR products from the first reaction were carefully handled to avoid cross-contamination. Three additional reactions were performed using the first product as the template. All reactions were performed in the presence of a negative and a positive control for *P. facilarum*, *P. vivax* and *P. malariae*. Images of gels representing all three reactions are presented in Figure 17. All samples included in this project were positive for *P. vivax*, and there was no evidence of mixed infection from other malaria parasites.

Figure 17: A PCR used to identify malaria parasites, based on the 18S SSUrRNA Type A gene. A: Identification of *P. vivax* by nested PCR reaction using primers pairs Plu5/6 and Viv1/2. Samples were produced by amplification of DNA from blood spots from cases 1-10 with severe or non-severe malaria; also shown are *P. vivax* positive control (+) and negative control (-). B: Nested PCR reaction using primers pairs Plu5/6 and Fal1/2. Samples were produced by amplification of DNA from blood spots from patients 1-5, and *P. falciparum* positive control (+) and negative control (-). C: Nested PCR reaction using primers pairs Plu5/6 and Mal1/2. Samples were produced by amplification of DNA from blood spots from patients 1-7, and *P. malariae* positive control (+) and negative control (-). Electrophoreses were performed on 1.5% agarose gel in Tris acetate-EDTA buffer and stained with ethidium bromide. M: 100 base pairs DNA marker.

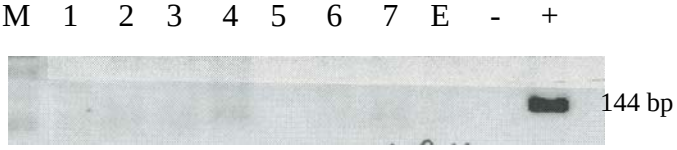
A. Identification of *P. vivax*:



B. Identification of *P. falciparum*:



C. Identification of *P. malariae*:

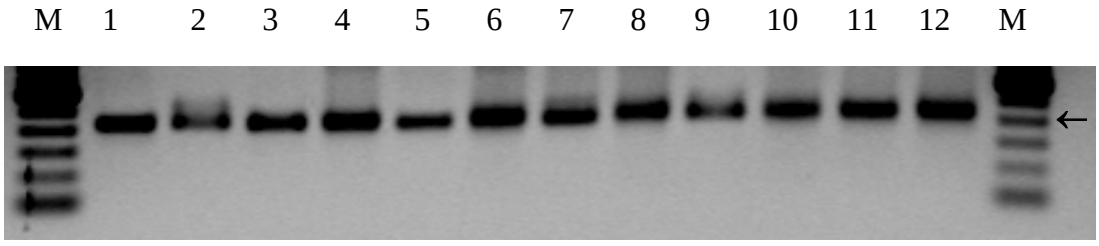


3.2.3 18S SSUrRNA Type A gene:

3.2.3.1 Standardization and Use of the Nested PCR to Amplify the 18S SSUrRNA Type A gene:

The PCR developed to study the segment between variable regions 7 and 8 of the 18S SSUrRNA Type A gene was successfully standardized and applied to amplify the *P. vivax* DNA present in the 32 samples included in this project (Figure 18). All PCR products were consistent in electrophoretic migration rate with the expected size, of approximately 450 base pairs. Examination of the gel images provided no indication of mixed alleles or clones; all products presented as single bands that were purified and sequenced. All products were sequenced at least twice using forward (Var1 and/or Var3) and reverse (Var2 and/or Var4) primers. Text files from the sequences were edited and combined in a consensus representing the region of interest. Conflicts were resolved by repeating the PCR and sequence reactions. Alignments were created and saved in appropriate formats for further phylogenetic analysis.

Figure 18: Image of agarose electrophoresis gel of amplified products obtained by nested PCR targeting the segment between variable regions V7 and V8 in the 18S SSUrRNA Type A gene using primer pairs Var1/2 and Var3/4. Lanes 1-12: sample DNA, M: 100 base pairs marker. Electrophoresis was performed on 1.5% agarose gel in Tris acetate - EDTA buffer stained with ethidium bromide.



3.2.3.2 Phylogenetic Analysis of the 18S SSUrRNA Type A gene sequences:

Strains from Manaus and references Salvador I and Belém:

Sequences of amplified PCR products of the 18S SSUrRNA Type A gene were edited to yield sequences of the same gene segment of 398 bases in length. Sequences of 32 samples from Manaus were aligned with sequences of the references strains, Salvador I (SALPAT) and Belém (BELPAT) (Annex Alignment 1). Determination of nucleotide compositions and phylogenetic analyses were performed.

Strains from Manaus had an average nucleotide composition represented by 35.5% thymidine (T), 17.7% cytosine (C), 27.2% adenine (A) and 19.6% guanine (G). There was no significant difference between strains.

A comparison of the two references strains Salvador I (SALPAT) and Belém (BELPAT) revealed one variable site at position 117: SALPAT presented a T and

BELPAT an A. There were twelve variable sites among the strains from Manaus. The A/T polymorphism at position 117 was noted, eighteen sequences had a T and fourteen had an A.

A T/C polymorphism was observed at position 100; both reference strains presented a T at this residue. Ten strains from Manaus had a C; five were obtained from patients admitted to the hospital for treatment and five from the outpatient group. The remaining twenty-two strains had a T at position 100; six were in the admitted group while sixteen were in the outpatient group.

In the other ten variable sites, mutations were present in one, two or three strains. At residue 33, an A→T transversion occurred in strain 361A. At position 58, strains number 738 and 827 presented a T by C substitution. At residue 67, strain number 126A presented an A→G transition.

At residue 75, strains C and 243A, had a transition of cytosine to thymidine. Strain C had point mutations identified in three additional sites: at residue 124, a cytosine was replaced by an adenine; at position 301, another cytosine was substituted by a thymidine, and at residue 336, a thymidine was replaced by adenine. The later mutation was also present in strain number 243A.

At position 118 in the alignment, strain number 66 had an A→G transition. At residue 119, the strains identified by the letters A and J presented a cytosine by thymidine transition. Three strains in the Admitted group of patients (D, F and G) had a T→C transition at residue number 352.

Of those strains presenting T at position 117, ten were obtained from patients admitted at the hospital for treatment and eight were from patients treated in the

outpatient clinic. For strains with the A polymorphism, one sequence was from a patient in the group treated at the hospital and thirteen were isolated from individuals treated as outpatients. Cross-tabulation of the data and a X^2-test revealed a statistical significant difference ($p<0.05$) between treatment groups based on frequency of A and T at position 117. The frequency of T in the admitted group was more than twice that in the outpatient group (Table 2).

Table 2: 18S SSUrRNA Type A gene, "A/T" polymorphism at position 117 present in strains from Manaus, by patient treatment group:

Patient treatment group	Position 117 of the Alignment		Total
	A	T	
Admitted	1	10	11
Outpatient	13	8	21
Total	14	18	32

X^2-test: $p<0.05$

To infer the phylogeny of *P. vivax* using the sequence data of the 18S SSUrRNA type A gene, distance and parsimony-based approaches were used. Reliability of the groups formed was tested using bootstrap analysis. Sequences from strains from Manaus and the reference sequences Salvador I (SALPAT) and Belém (BELPAT) were aligned and rooted by the outgroup method with *P. cynomolgi*.

In Figure 19, a representative phylogenetic tree is presented. In this analysis, the method of heuristic search, with the optimality criterion set to parsimony, was used. Four clusters, based on different combinations of the polymorphisms found at residues 100 and 117, are represented in this tree. Thirteen strains had 100:T and 117:A, nine strains had 100:C and 117:T, one strain had 100:C and 117:A and nine strains had 100:T and 117:T.

In the 100:T/117:A group (font in pink) strains B, 131, 136, 185, 321, 340, 442, 913, 95A and 216A had sequences identical to the Belém strain, however, variable sites in strains 738, 126A and 361A distinguished them within that group. In the 100:C/117:T group (font in green) strains 212, 294B and 297A shared the same sequence, only differing from the Salvador I strain by the T/C polymorphism at position 100; strains D, F and G differed from this group in the addition of a mutation at position 352. Strains C and 243A shared the mutation at residue 336 but differed in other sites. Variation in residue 119 separated strain A from the group.

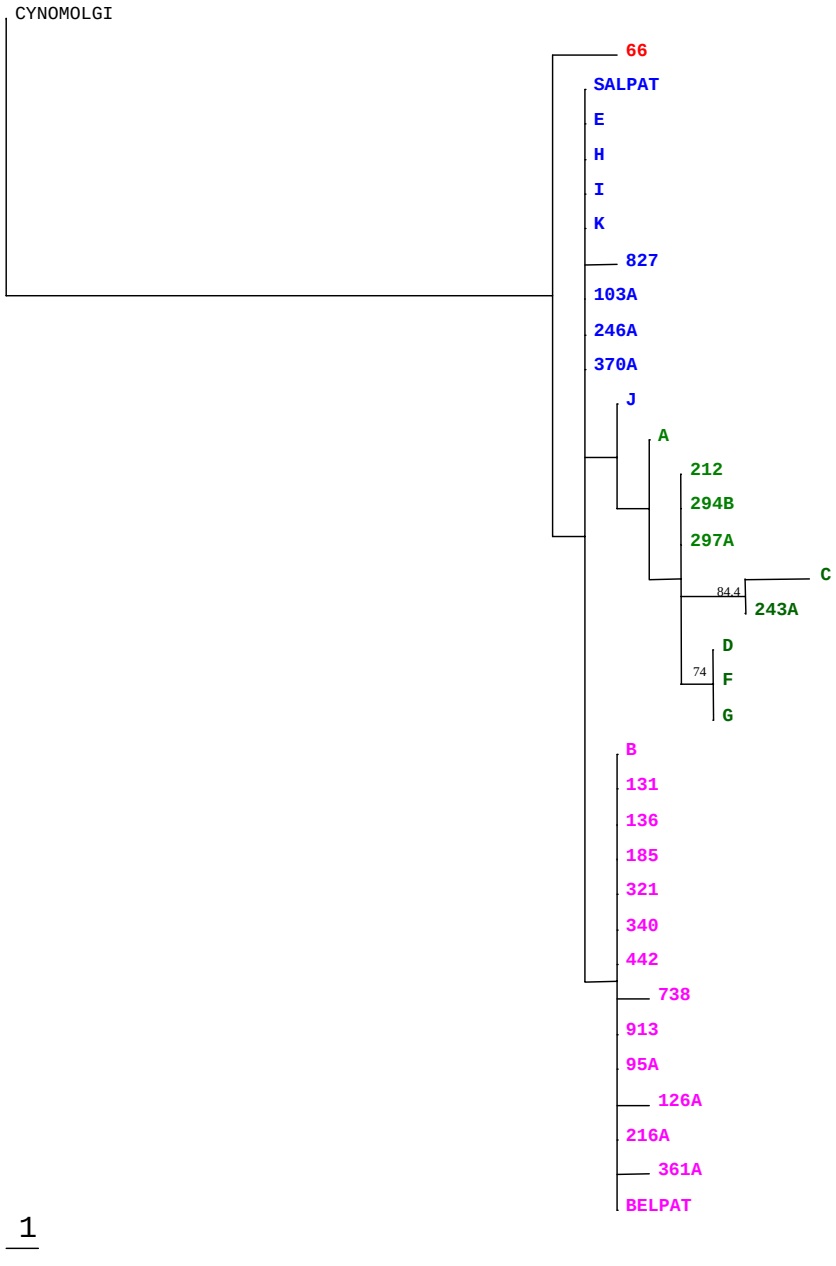
In the 100:T/117:T group (font in blue), strains identified by E, H, I, K, 103A, 246A and 370A were identical to the Salvador I strain. Strain number 827 had an additional variable site at position 58 and strain J had a point mutation in residue 119; separating them from the rest of the group. Only one strain, number 66 (font in red), was identified in the 100C/117:A group; this strain had an additional variable site at position 118. Bootstrap support values were significant for the group composed of strains D, F and G (bootstrap value of 74.0%) and for the group composed of strains C and 243A (bootstrap value of 84.4%). Classification of the patients into four groups based on the 100/117 polymorphisms was statistically significant (Table 3).

Table 3: 18S SSUrRNA Type A gene, distribution of the strains based on the polymorphism at positions 100 and 117 and patient treatment group.

Patient treatment group	100/117 polymorphism				Total
	100T/117A	100T/117T	100C/117T	100C/117A	
No relapse	8	1	1	1	11
Relapse	4	3	3	0	10
Admitted	1	5	5	0	11
Total	9	9	9	1	32

X^2-test: $p<0.05$

Figure 19: Heuristic search tree showing inferred phylogeny of 18S SSUrRNA Type A gene of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELPAT) rooted with *P. cynomolgi*. Heuristic search tree constructed under the optimality criterion of parsimony; starting tree obtained by stepwise addition, sequences added at random, tree-bisection-reconnection used as the algorithm for branch swapping. Colors represent different groups based on polymorphisms at residues 100 and 117. Numbers are bootstrap support values.



Comparison of Sequences of Study Strains to Sequences Available in Gene Bank:

Sequences corresponding to the same segment of the SSUrRNA gene that we determined for our strains were available for four additional strains in Gene Bank: one from Colombia, one from Thailand and two sequences from El Salvador (Table 4). These sequences were aligned with the strains from Manaus (Annex Alignment 1) and used to further define the phylogeny of the study strains.

The thymidine at position 117 that differentiates the Salvador I strain from the Belém strain was also present in both Gene Bank sequences from El Salvador. With respect to the polymorphism at position 100, ELSAL was T and SALI was C at the residue. Additional point mutations were noted in both El Salvador strains, both T→C transitions, ELSAL at residue 58 and SALI at position 229 in the alignment. The Gene Bank sequences from Colombia and Thailand were similar to the Belém strain, with A at position 117.

Table 4: 18S SSUrRNA Type A gene sequences obtained from Gene Bank:

Access Number	Isolate	Year	Country	Reference
L07559	*P. cynomolgi* Vietnamese strain	1993	Origin: Vietnam	Waters et al., 1993
U93233	Pvk1294 – PVTHAI	1997	Thailand	Li et al., 1997
U83877	P595-42 – COLOMBIA	1997	Colombia	Montoya et al., 1997
U07367	ELSAL	1994	El Salvador	Li et al., 1994
U03079	SALI	1993	El Salvador	Qari et al., 1994

To infer the phylogeny of *P. vivax* using the sequence data of the 18S SSUrRNA Type A gene, distance and parsimony-based approaches were used. The reliability of the groups formed was tested using bootstrap analysis. Sequences from the Gene Bank were

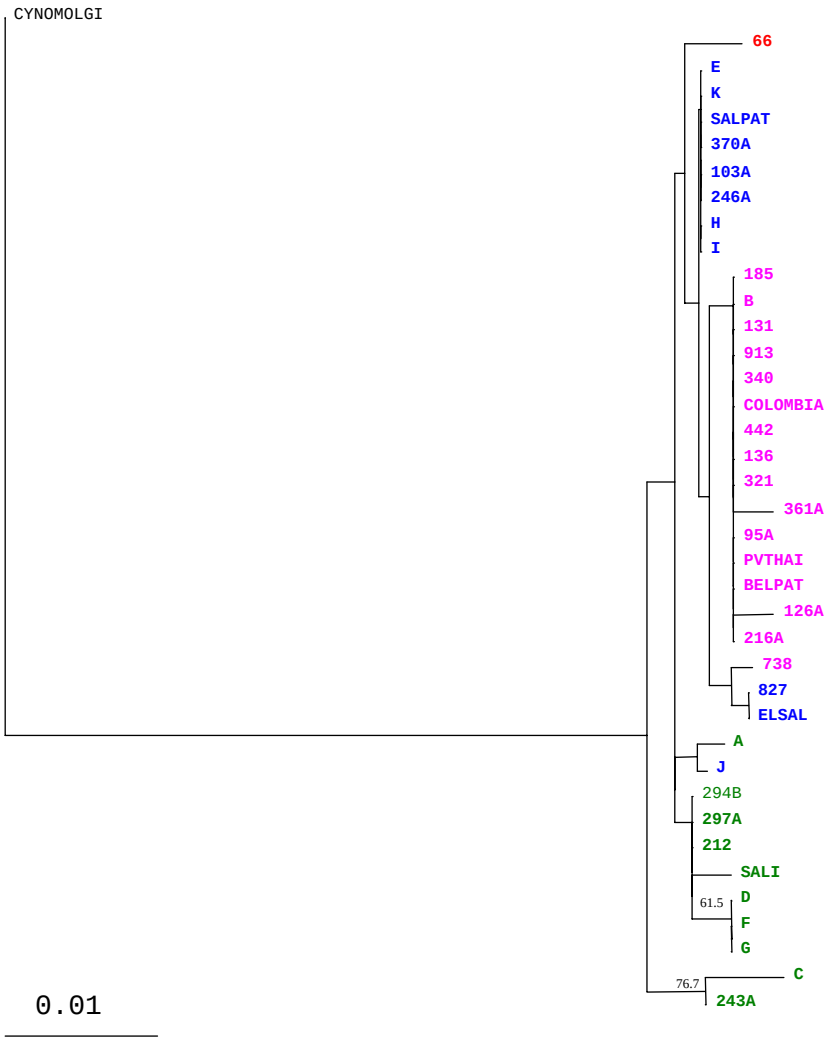
included in the previous alignment containing strains from Manaus. The reference sequences Salvador I and Belém and rooted by the outgroup method with *P. cynomolgi*.

Figure 20 shows a representative tree constructed with the Neighbor-Joining method. Bootstrap analysis was performed with the cutoff set to 50%, values above this cutoff were posted in the nodes of the tree. The strains are color coded according to the polymorphisms observed at residues 100 and 117: pink for 100T/117A, red for 100C/117A, green for 100C/117T, and blue for 100T/117T. The strains from Colombia and Thailand (PVTHAI) were grouped with the strains in the 100:T/117:A, as they shared the same sequence as the reference strain Belém.

ELSAL (100:T/117:T) occupied the same branch with strains number 738 (100:T/117:A) and 827 (100:T/117:T). They presented a T→C transition at position 58; the formation of this group was not supported by bootstrap analysis (bootstrap support value of 53.2%). SALI had the 100C/117T sequence and was grouped with strains from Manaus presenting the same characteristic. The strains identified by the letters A (100:C/117T) and J (100:T/117T) were grouped together and became neighbors with the group 100:C/117:T. Both strains presented a thymidine at residue 119 but this topology was not supported by bootstrap analysis (Support value of 52.8%).

Based on this analysis, the branch formed by strains D, F and G within the group characterized by 100:C/117:T residues had a support value of 61.5%. Also in this analysis, strains C and 243A formed their own branch, with a bootstrap support value of 76.7%.

Figure 20: Neighbor-Joining tree showing inferred phylogeny of the 18S SSUrRNA Type A gene of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELPAT), and strains from Gene Bank rooted with *P. cynomolgi*. Tree constructed under the optimality criterion of distance (minimum evolution) measured by the HKY 85 method (Hasegawa, Kishino, Yano, 1985). Colors represent different groups based on polymorphisms at residues 100 and 117. Numbers are bootstrap support values.



Minimum Evolution Score = 0.079
Sum of Branches length = 0.080

Strains from Patients with Two Time-Point Infections:

The DNA extracted from patients presenting infections at two time-points was amplified using primers for the SSUrRNA Type A gene. The aligned sequences are presented in Figure 21. Samples 126A and 126B differed from each other at three residues, including the mutations 67 A/G, 100 T/C, and 117 A/T. Samples 361A and B differed from each other as a result of the transition T/A at residue 33. Therefore, it is likely that strains 126A and 126B, and possibly strains 361A and 361B, were different strains resulting from mixed or sequential infections. The sequences of each of the remaining pairs were identical in this region, and may have reflected relapse of the original infection.

When the sequences from strains 126B and 361B were included in the overall alignment, we observed that the nucleotide composition was identical to others in the alignment. From the 100:C/117:T group, strain 126B was identical to 297A. The construction of their phylogenetic trees using distance and parsimony criteria placed them together in the same branch. Samples 361B, 95A, 131, 913 and B were grouped in the same branch, as they were identical.

Figure 21: 18S SSUrRNA Type A nucleotide sequence alignment of strains from patients that presented two time-point infections during the treatment for *P. vivax*. Identical nucleotides are represented by dots, point mutations are presented in bold upper cases, and polymorphisms at residues 100 and 117 are highlighted in yellow.

```
95A  TCTGGTTAAT TCCGATAACG AACGAGATCT TAACCTGCTA ATTAGCGGCA AATACGATAT ATTCTTACGT GGGACTGAAT TCGGTTGATT TGCTTACTTT  [100]
95B  .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
103A .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
103B .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
126A .......... .......... .......... .......... .......... .......... ......G... .......... .......... .........  [100]
126B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........C  [100]
216A .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
216B .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
246A .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
246B .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
294A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........C  [100]
294B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........C  [100]
361A .......... .......... .......... ..T....... .......... .......... .......... .......... .......... .........  [100]
361B .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
370A .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
370B .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
KA   .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]
KB   .......... .......... .......... .......... .......... .......... .......... .......... .......... .........  [100]

95A  GAAGAAAATA TTGGGAAACG TAACAGTTTC CCTTTCCCTT TTCTACTTAG TTCGCTTTTC ATACTGTTTC TTTTTCGCGT AAGAATGTAT TTGCTTGATT  [200]
95B  .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
103A .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
103B .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
126A .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
126B .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
216A .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
216B .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
246A .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
246B .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
294A .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
294B .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
361A .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
361B .......... ......A... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
370A .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
370B .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
KA   .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]
KB   .......... ......T... .......... .......... .......... .......... .......... .......... .......... ..........  [200]

95A  GTAAAGCTTC TTAGAGGAAC GATGTGTGTC TAACACAAGG AAGTTTAAGG CAACAACAGG TCTGTGATGT CCTTAGATGA ACTAGGCTGC ACGCGTGCTA  [300]
95B  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
103A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
103B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
126A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
126B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
216A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
216B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
246A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
246B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
294A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
294B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
361A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
361B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
370A .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
370B .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
KA   .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]
KB   .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [300]

95A  CACTGATATG TATAACGAGT TATTAAAATT ACGATTCAGC TTGCTGTTTC GTATTTTTCC TCCACTGAAA AGTGTAGGTA ATCTTTATCA GTACATAT [398]
95B  .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
103A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
103B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
126A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
126B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
216A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
216B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
246A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
246B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
294A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
294B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
361A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
361B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
370A .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
370B .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
KA   .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
KB   .......... .......... .......... .......... .......... .......... .......... .......... .......... ........ [398]
```

3.2.4 CSP gene:

3.2.4.1 Standardization and Use of the PCR and RFLP Analysis Targeting the CSP gene:

Initial experiments demonstrated that errors in amplification in the repeat variable region occurred when regular *Taq* polymerase was used in the PCR reactions. These errors produced grossly aberrant sequences of the amplicons.

To improve fidelity and reduce the incidence of *in vitro* artifacts introduced by *Taq* polymerase, we modified the protocol to use enzymes with 3'-5' exonuclease, or proof reading, activity (*rTth* polymerase and high fidelity Hot-start polymerase).

Plasmid DNA was used as a template for standardizing the PCR. The primers selected were able to amplify the DNA of the three *P. vivax* variants, generating products of slightly different sizes (Figure 22) and amplifying the DNA of the reference strains Salvador I (SALPAT) and Belém (BELEM).

Primers N1 and N2 were used as internal primers in the CSP nested PCR reaction. We later modified the protocol for a semi-nested reaction using primer pairs N1 and P2 to obtain extended sequence reads from the conserved regions I and II.

There were 32 patients included in the study, eleven of which were treated in the hospital and 22 were treated as outpatients in the ambulatory clinic. Ten patients in the ambulatory group presented a second time-point infection during treatment for malaria; CSP gene sequences from eight of these patients were amplified by PCR. One patient treated in the hospital also presented a second time-point infection and the corresponding DNA was amplified by PCR.

When the PCR products were analyzed by electrophoresis, sample C in the admitted group and sample 126B in the relapse group presented double bands. PCR products from sample C were cloned. Sequencing of different clones identified a mixed infection with variants *P. vivax* VK210 and *P. vivax*-like. The two bands from Sample 126B were separated by cutting the gel and extracting the products. The results identified both products as *P. vivax* VK210, but with different numbers of repeat units.

The enzymes selected for the RFLP analyses were used in the specific differentiation of the three CSP variants. As shown in Figure 23A, the enzymes PVU II, Bcl I and BstX I selectively digested the VK210, VK247 and *vivax*-like DNA. The protocol was used to screen the PCR DNA from Manaus strains, as exemplified by the results shown in Figure 23B.

A few DNA samples were not digested by any of the three selected restriction enzymes, as exemplified by samples 212 and 66 in Figure 23B. Sequencing of these products revealed that they belonged to the VK210 group but had lost the restriction site for PVUII as result of mutations. None of the DNA samples was digested by the enzymes Bcl I or BstX I, except for the larger band from sample C which was digested by Bcl I.

Figure 22: Standardization and application of the PCR protocol to amplify and distinguish *P. vivax* CSP types. A: Nested PCR results of amplification of CSP gene segments from plasmids containing the *P. vivax* VK210, VK247 and *vivax*-like CSP genes. First round amplification was performed using P1 and P2 primers and second round reactions were performed using N1 and N2 primers. B: Semi-nested PCR products obtained from study samples using the P1 and P2 and N1 and P2 primers for first PCR and second round reactions, respectively. Lane M contained 100 bp markers. Remaining lanes contain DNA amplified from study samples, as indicated. Electrophoreses were performed on 1.5% agarose gel in tris acetate EDTA buffer stained with ethidium bromide.

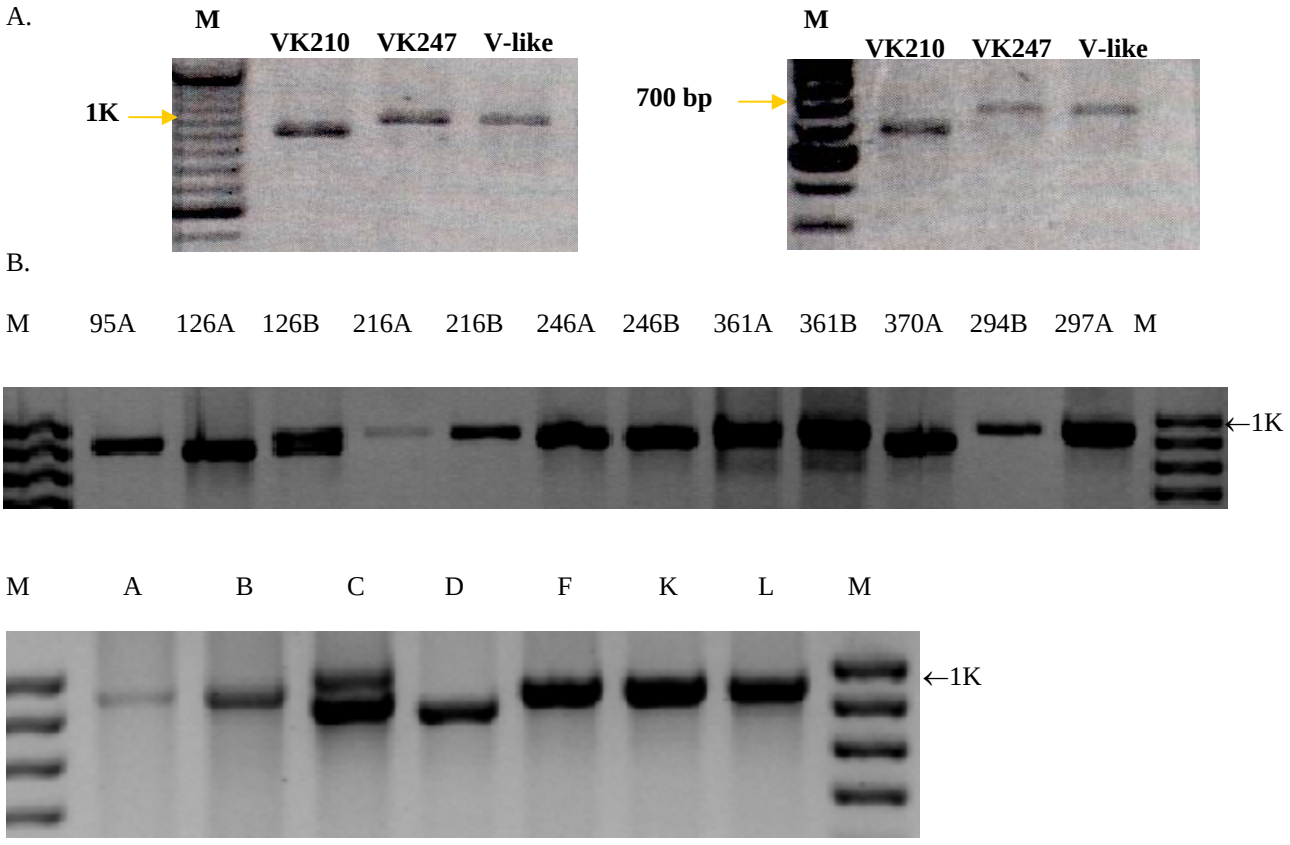
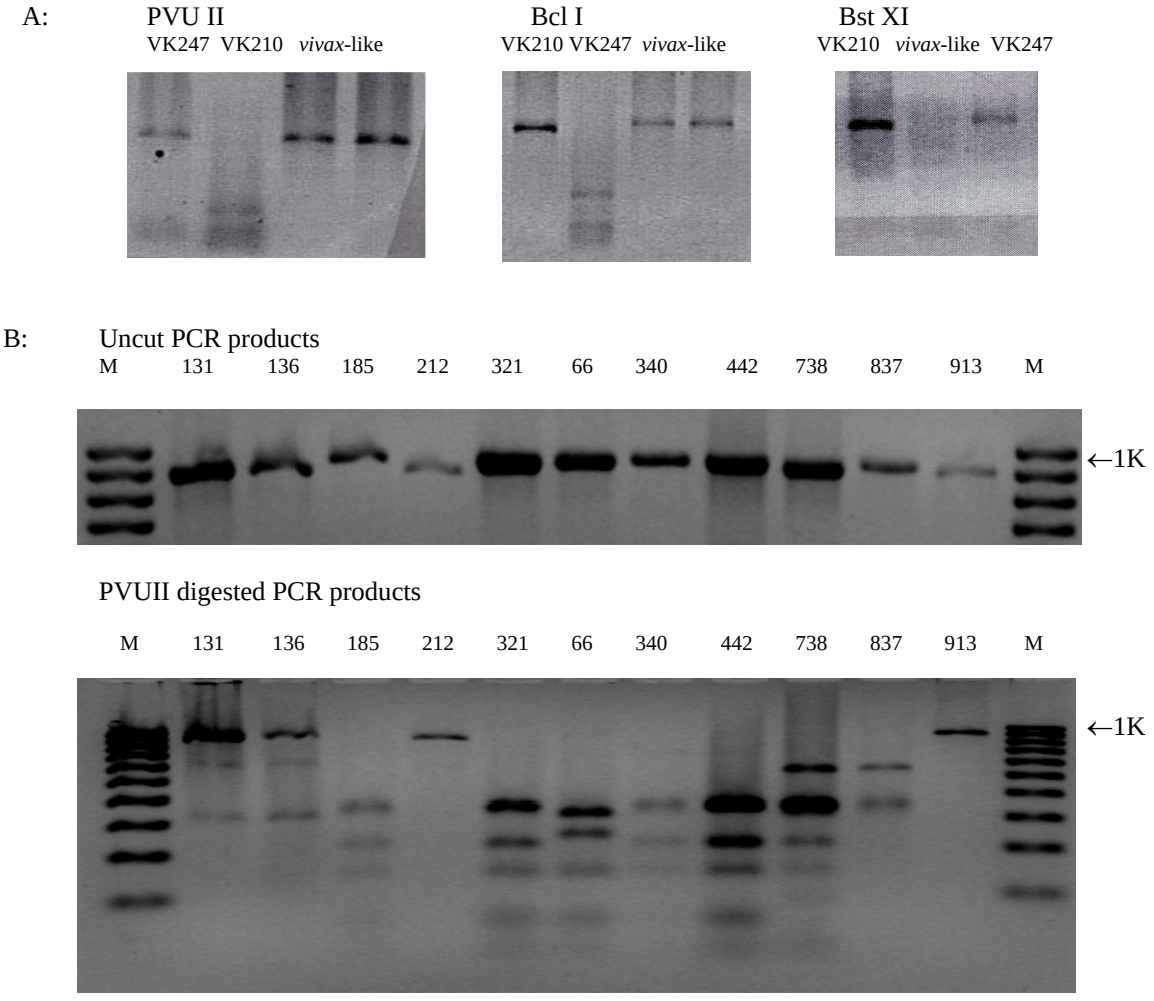


Figure 23: Analysis of *P. vivax* CSP types using RFLP of PCR products. A: RFLP analysis of nested PCR products obtained using plasmids containing VK210, VK247, and *vivax*-like CSP genes. PCR products were digested with PVU II, Bcl I and BstX I. B: Semi-nested PCR products obtained from study samples using the P1/P2 and N1 and P2 primers for first PCR and second round reactions, respectively, followed by RFLP. Lane M contained 100 bp markers. Remaining lanes contained DNA amplified from study samples, as indicated. Electrophoreses were performed on 1.5% agarose gel in tris acetate EDTA buffer stained with ethidium bromide.



3.2.4.2 Phylogenetic Analysis of the CSP gene Sequences:

Forward and reverse sequences were assembled and the contigs were edited and aligned using Megalign 3.06b (DNASTAR Inc.) and Clustal X 1.83. Gaps were edited manually to avoid the separation of nucleotides from the same codons or repetitive blocks. DNA sequences were translated to amino acid using EditSeq 3.88 (DNASTAR Inc.). Amino acid alignments were used to classify the *P. vivax* VK210 sequences, based on number and type of peptide repeats.

To study the phylogeny of *P. vivax* based on the CSP gene, nucleotide and amino acid sequence alignments were prepared combining sequence data from samples, reference strains Salvador I and Belém, and Gene Bank sequences. Analyses were performed with and without the outgroup root, *P. yoelii*.

Strains from Manaus compared to references Salvador I and Belém:

The sequences of the Manaus strains were compared to the sequences of the reference strains Salvador I and Belém. Dr. Barnwell of the Centers for Disease Control (CDC, Atlanta, GA) provided genomic DNA from Salvador I and Belém strains. PCR products obtained using these genomic DNA were sequenced and compared to sequences available from the Gene Bank. Our Salvador I strain sequence was completely identical to the Sal-I sequence with accession number J02751, but our Belém strain sequence had an amino acid deletion in repeat block 21 when compared to the sequence with accession number M11926.

a. Nucleotide Composition:

A primary analysis was performed on 32 DNA samples, eleven from patients admitted to the hospital for treatment and 21 from patients treated in the outpatient clinic.

Variation in the variable repeat region resulted in products of different sizes. The number of nucleotides per comparable gene segment ranged from 791 and 845. The strains from Manaus had 11.7% thymidine (T), 18.9% cytosine (C), 36.5% adenine (A) and 32.8% guanine (G).

At the codon level, the first position had a predominance of G (57.6% average); the second codon position was almost evenly composed of C (31.2%), A (33%) and G (29.7%), and the third position was predominantly composed of A (56.3%). There were 781 constant characters and 70 variable characters, 12 of which were parsimony-uninformative and 58 of which were parsimony-informative. There were an average of 796 identical pairs over all taxa, 9 transitional (si) pairs, 10 transversional (sv) pairs and an average transition/transverion rate (d) of 0.9.

b. Translated Amino Acid Sequences and Classification of P. vivax CSP VK210:

The CSP is composed of two conserved regions flanking a variable region. The variable region contains repeating blocks, each of which is composed of nine amino acids. The variable region of the CSP genes of the reference strains Belém and Salvador I are both composed of 21 variable repeat blocks. The amino acid sequences of each of the first 18 repeat blocks of the Belém and Salvador I strains are either GDRADGQPA or GDRAAGQPA (Figure 24).

The two strains were discordant at the fifth position of the repeats that formed the first 18 repeats. Blocks 19, 20 and 21 contained additional polymorphisms that distinguished the two reference strains. The sequence of block 20 of the Salvador I strain was GDRAAGQAA, while that of the Belém strain was GNGAGGQAA. A comparison

between our Belém strain (BELPAT) and the Gene Bank strain, accession number M11926, revealed a deletion of one amino acid at repeat block 21.

Figure 24: CSP amino acid sequence alignment of the reference strains Salvador I and Belém. Identical amino acids represented by dots and gaps represented by dashes.

```
BELEM  KDGKKAEPKNPRENKLKQP GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GDRADGQPA
BELPAT ................... ......... ......... ......... ......... .........
SALPAT ................... ......... ......... ......... ....D.... ....A....

BELEM  GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA
BELPAT ......... ......... ......... ......... ......... ......... .........
SALPAT ......... ......... ......... ....D.... ......... ....A.... ....D....

BELEM  GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRAAGQPA GDRAAGQPA GDRAAGQPA
BELPAT ......... ......... ......... ......... ......... ......... .........
SALPAT ....A.... ....D.... ....A.... ....D.... ......... ......... .......A.

BELEM  GNGAGGQAA GGNAGGGQGQ NNEGANAPNEKSVKEYLDKVRATVGTEWTPCSVTCGVGVRVRRR
BELPAT ......... ......-... ........................................
SALPAT .DR.A.... ......-... ........................................

BELEM  NAANKKPEDLTLNDLETDVCTMDKCAGI
BELPAT ............................
SALPAT ............................
```

Among the 32 test strains, there were 12 unique amino acid sequences identified, as shown in Figure 25 and Table 5. Considerable variation in amino acid sequences was noted among the strains and test samples. An alanine insertion was noted in three test samples, C, E and 126A, at the position immediately before the first repeat block. A highly conserved repeat block motif, GDRAD/AGQPA, was noted in each case. This motif with or without single point mutations accounted for the first 17, 18 or 19 of the repeat blocks in all cases.

The Belém and Salvador I strains differed at the fifth position in nine of the conserved repeat blocks. The sequences corresponding to block 19 and 20 varied from the conserved motif in all strains, except for test sample 95A, in which the sequence corresponding to block 20 was deleted.

Block 20 consisted of the sequence GNGAGGQAA in all cases, except for the Salvador I strain and test samples 66, 361A, and K. These strains shared common mutations at the second, third and fifth position of this block. Strain 66 had an additional mutation in block 21, changing the amino acid at position nine from alanine to proline. The sequence corresponding to block 21 was also highly conserved, and consisted of the sequence GGNAGGQGQ, except in the case of the Belém strain (M11926) from Gene Bank, which had an insertion mutation of a G at the seventh position.

Among the test samples, seven had a deletion of one conserved repeat blocks, and eight had deletions of two conserved repeat blocks. The most common point of variability among the test samples was the D/A polymorphism at position five of the conserved repeat blocks. Besides these polymorphisms, sample K had mutations at position eight of conserved blocks 16, 18 and 19. Samples I, D, and 95A had mutations at position 2 in block 6; strain 95A had a mutation at position eight in block 18.

Table 5: Classification of the strains from Manaus according to *P. vivax* CSP VK210 sequence and patient treatment group.

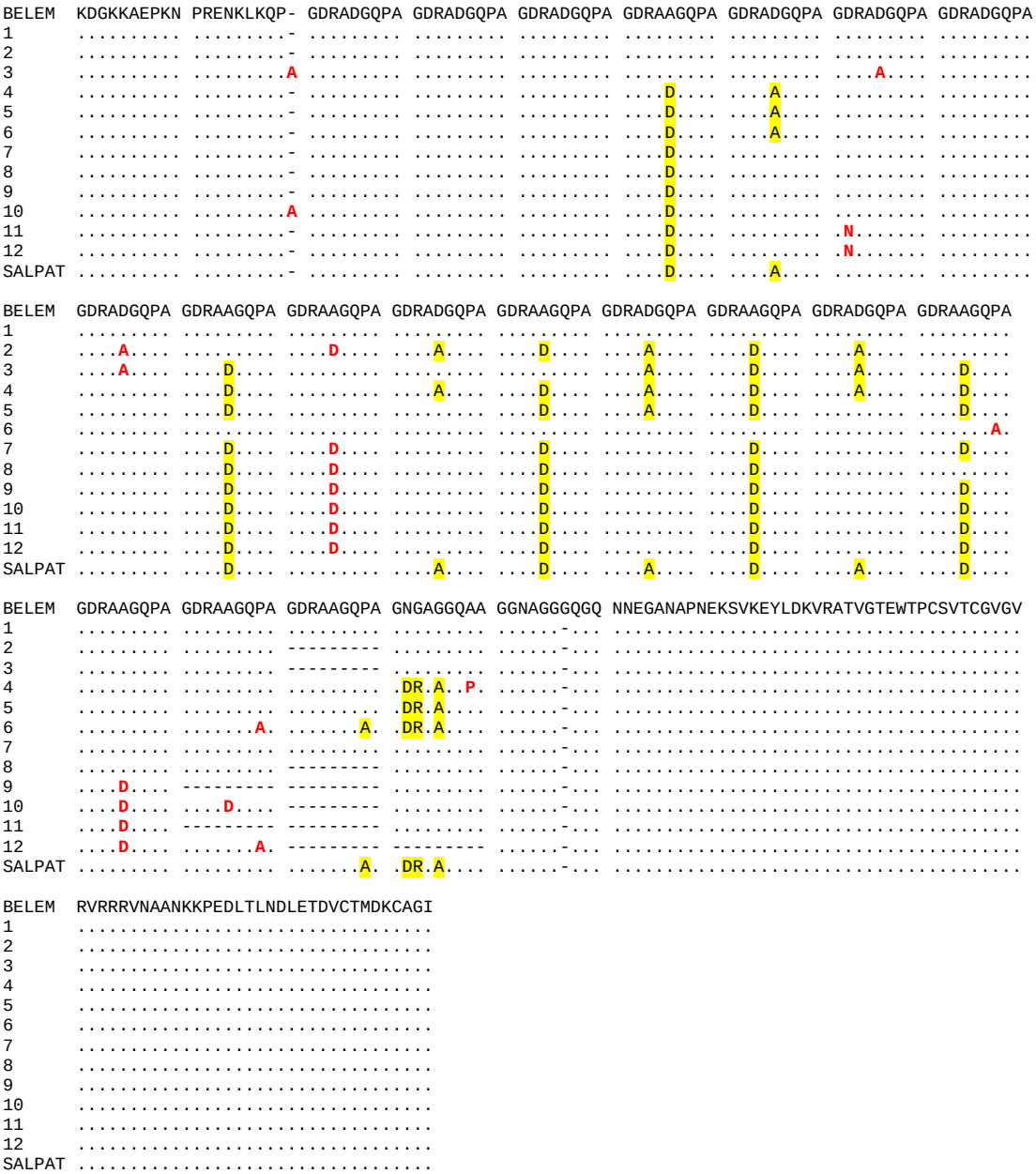
Sequence groups	Patient Treatment Group		
	No relapse	Relapse*	Admitted
1	185, 321, 340, 442	216A, 243A, 294B**, 297A	A, B, F, H, J
2			G
3			C♣, E
4	66		
5		361A	
6			K
7		246A	
8	738, 827	103A	
9	131, 136, 212, 913	370A	
10		126A	
11			I, D
12		95A	

* First-time infection sample represented

** Does not have PCR product for the first-time infection

♣ One representative clone

Figure 25: Alignment of deduced amino acid sequences of CSP gene segments from study samples and reference strains Salvador I and Belém. The unique sequences found in test samples are shown. Identical amino acids are represented by dots and gaps represented by dashes.



As shown in Table 5, test samples from patients admitted to the hospital for treatment, were distributed among five different sequence groups, including three that were considered Belém-like.

Among the 12 unique sequences, seven were found in a single test sample, two were found in two test samples, one was found in three test samples, one was found in five test samples, and one was found in 13. The sequence of the largest sequence group, group 1, differed from that of the Belém strain (M11926), in the insertion of a mutation in block 21 of the Belém strain. The second largest group, group 9, was also more closely related to the Belém strain than the Salvador I strain, based on the polymorphisms of repeat block 20.

Groups 4, 5 and 6, each consisting of single samples, were more closely related to the Salvador I based on the polymorphism at repeat block 20. Based on similarities and differences in the amino acid sequences, all the seven other amino acid groups were more related to the Belém strain.

Among the test samples in group 1, six had an A at nucleotide 117 in the SSUrRNA gene, and seven had a T. Similarly, among all samples that were Belém-like, six were A and ten were T at this residue. Among the 10 samples that had been found to have C at nucleotide 100 in the 18S SSUrRNA Type A gene, five were in group 1, four were considered Belém-like and one was Salvador I-like based on CSP sequences.

Strains from Second Time-Point Infection from Patients Treated in the Outpatient Clinic:

In the samples selected for this study, 21 originated from patients treated as outpatients in the malaria clinic of the FMT-Am in Manaus, Amazonas. In ten of these patients, malaria parasites were detected at a second-time point during treatment. Seven of the ten patients had CSP genes amplified and sequenced using forward and reverse primers. In one sample, 126B, there was a mixed infection represented as double bands in the agarose gel; these bands were separated by gel extraction and sequenced. One patient in the group admitted for treatment to the hospital, sample K, also had a second time-point infection. This patient's second DNA sample was also submitted to PCR and sequencing of the CSP gene.

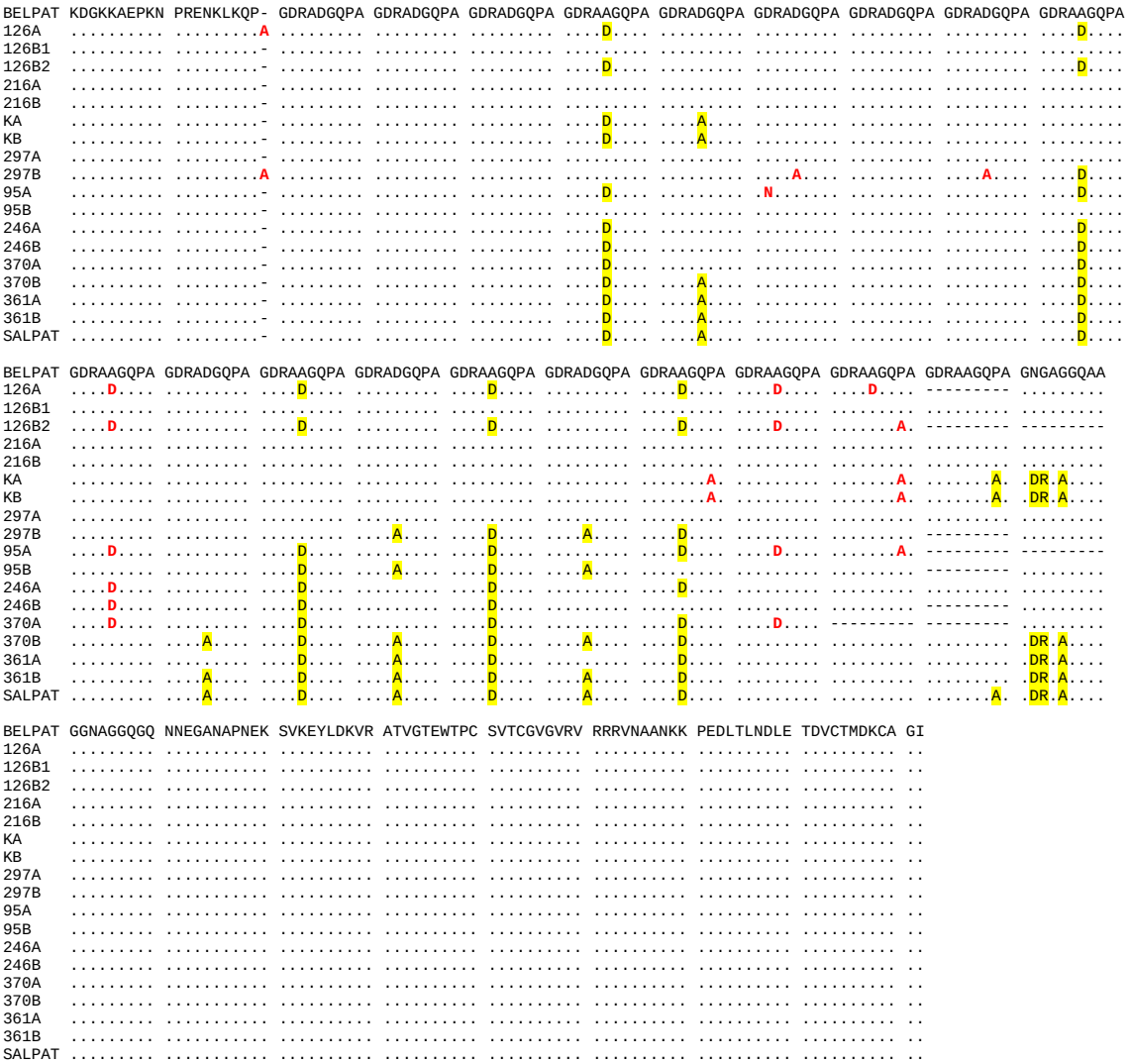
DNA sequences were edited, translated to amino acid and aligned using Belém and Salvador I strains as references (Figure 26). Six of the eight pairs presented divergence between the amino acid sequences. The letter "A" after patient identification code indicates that the strain was isolated from the first time-point infection, and "B" represents a second time-point infection. Amino acids unique to the strains from Manaus are presented in red, while those identical to the Salvador I strain are highlighted in yellow.

Amino acid sequences from strains isolated from patients K and 216 were identical. All strains isolated from first and second time-point infections of the patients identified as 126, 297, 95, 246, 370, and 361 were divergent. The differences included point mutation changing amino acids, insertion of codons, and the deletion of entire blocks. Strains originating from both time-point collections from samples 126, 297, 95,

246 and 370 diverged not only in amino acid sequence but also in the number of repeat blocks that varied between 19 and 21. Both strains isolated from samples 361A and 361B presented 21 amino acid blocks and repeat block 20 presented the amino acid motif characteristic of the Salvador I strain. Divergence was found in the fifth position of blocks 11 and 15 where aspartic acid (D) present in strain 361A was replaced by alanine (A) in strain 361B.

Nucleotide sequence (Annex Alignment 3) was compared among the early and late sequences from samples KA/KB and 216A/216B which presented identical amino acid sequences. There was 100% agreement in the sequences from strains KA and KB but DNA sequences from strains 216A and 216B diverged at residue 132. Sample 216A presented an adenine while 216B presented a guanine.

Figure 26: CSP amino acid sequence alignment of strains from patients that presented two time-point infections during treatment for *P. vivax*. Belém (BELPAT) was the reference strain. Identical amino acids are represented by dots, and gaps are represented by dashes. Amino acids unique to strains from Manaus are shown in red, amino acids identical to Salvador I (SALPAT) are highlighted in yellow.



Strains from Manaus Considering the New Strains Identified from Second Time-Point Infections:

Strains isolated from the second time point infection were incorporated into the amino acid alignment of the existing data set (Alignment annexed). All strains were compared to each other and to the reference strains Salvador I and Belém. Three additional unique sequences were identified.

Distribution of the strains by VK210 sequence and treatment groups are presented in Table 6. Two late samples presented amino acid sequences identical to those in group 1, 126B-1 and 216B. Strain KB was 100% identical to KA in amino acid and nucleotide sequences. Amino acid sequences from two new sequence groups, 13 and 14, were each present in one test sample and amino acid sequence group 15 was present in two test samples.

In group 13, the fifth position of amino acid blocks 10 and 17 presented point mutations resulting in the substitution of an alanine (A) with an aspartic acid (D), and the deletion of repeat blocks 19 and 20 (Figure 27). The repeat blocks found in sample 95B included GNGAGGQAA in repeat block 20, relating this strain to Belém-group. Group 15, composed of samples 361B and 370B, had 21 repeat blocks and included GDRAAGQAA, characteristic of the Salvador I strain.

Table 6: New classification of the strains from Manaus according to *P. vivax* CSP VK210 sequence and patient treatment group.

Sequence groups	Patient Treatment Group		
	No relapse	Relapse	Admitted
1	185, 321, 340, 442	126B-1, 216A, 216B, 243A, 294B**, 297A	A, B, F, H, J
2			G
3		297B	C♣, E
4	66		
5		361A	
6			KA, KB
7		246A	
8	738, 827	103A, 246B	
9	131, 136, 212, 913	370A	
10		126A	
11			I, D
12		95A	
13	126B-2		
14	95B		
15		361B, 370B	

** Does not have PCR product for the first-time infection

♣ One representative clone

Figure 27: New deduced amino acid sequences of CSP gene segments from study samples and references Belém and Salvador I. Identical amino acids presented by dots, unique amino acids in red, similarities to Salvador I in yellow, gaps shown as dashes.

```
BELEM  KDGKKAEPKN PRENKLKQP- GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRADGQPA GDRADGQPA
1      .......... .........- ......... ......... ......... ......... ......... ......... .........
2      .......... .........- ......... ......... ......... ......... ......... ......... .........
3      .......... .........A ......... ......... ......... ......... ......... ....A.... .........
4      .......... .........- ......... ......... ......... ....D.... ....A.... ......... .........
5      .......... .........- ......... ......... ......... ....D.... ....A.... ......... .........
6      .......... .........- ......... ......... ......... ....D.... ....A.... ......... .........
7      .......... .........- ......... ......... ......... ....D.... ......... ......... .........
8      .......... .........- ......... ......... ......... ....D.... ......... ......... .........
9      .......... .........- ......... ......... ......... ....D.... ......... ......... .........
10     .......... .........A ......... ......... ......... ....D.... ......... ......... .........
11     .......... .........- ......... ......... ......... ....D.... ......... .N....... .........
12     .......... .........- ......... ......... ......... ....D.... ......... .N....... .........
13     .......... .........- ......... ......... ......... ....D.... ......... ......... .........
14     .......... .........- ......... ......... ......... ......... ......... ......... .........
15     .......... .........- ......... ......... ......... ....D.... ....A.... ......... .........
SALPAT .......... .........- ......... ......... ......... ....D.... ....A.... ......... .........

BELEM  GDRADGQPA GDRAAGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA
1      ......... ......... ......... ......... ......... ......... ......... ......... .........
2      ....A.... ......... ....D.... ....A.... ....D.... ....A.... ....D.... ....A.... .........
3      ....A.... ....D.... ......... ......... ......... ....A.... ....D.... ....A.... ....D....
4      ......... ....D.... ......... ....A.... ....D.... ....A.... ....D.... ....A.... ....D....
5      ......... ....D.... ......... ......... ....D.... ....A.... ....D.... ......... ....D....
6      ......... ......... ......... ......... ......... ......... ......... ......... .....A.
7      ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
8      ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... .........
9      ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
10     ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
11     ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
12     ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
13     ......... ....D.... ....D.... ......... ....D.... ......... ....D.... ......... ....D....
14     ......... ......... ......... ......... ....D.... ....A.... ....D.... ....A.... .........
15     ......... ....D.... ......... ....A.... ....D.... ....A.... ....D.... ....A.... ....D....
SALPAT ......... ....D.... ......... ....A.... ....D.... ....A.... ....D.... ....A.... ....D....

BELEM  GDRAAGQPA GDRAAGQPA GDRAAGQPA GNGAGGQAA GGNAGGGQGQ NNEGANAPNEKSVKEYLDKVRATVGTEWTPCSVTCGVGVR
1      ......... ......... ......... ......... ......-... ........................................
2      ......... ......... --------- ......... ......-... ........................................
3      ......... ......... --------- ......... ......-... ........................................
4      ......... ......... ......... .DR.A..P. ......-... ........................................
5      ......... ......... ......... .DR.A.... ......-... ........................................
6      ......... .......A. .......A. .DR.A.... ......-... ........................................
7      ......... ......... ......... ......... ......-... ........................................
8      ......... ......... --------- ......... ......-... ........................................
9      ....D.... --------- --------- ......... ......-... ........................................
10     ....D.... ....D.... --------- ......... ......-... ........................................
11     ....D.... --------- --------- ......... ......-... ........................................
12     ....D.... ........A. --------- --------- ......-... ........................................
13     ....D.... ........A. --------- --------- ......-... ........................................
14     ......... ......... --------- ......... ......-... ........................................
15     ......... ......... ......... .DR.A.... ......-... ........................................
SALPAT ......... ......... .......A. .DR.A.... ......-... ........................................

BELEM  VRRRVNAANK KPEDLTLNDL ETDVCTMDKC AGI
1      .......... .......... .......... ...
2      .......... .......... .......... ...
3      .......... .......... .......... ...
4      .......... .......... .......... ...
5      .......... .......... .......... ...
6      .......... .......... .......... ...
7      .......... .......... .......... ...
8      .......... .......... .......... ...
9      .......... .......... .......... ...
10     .......... .......... .......... ...
11     .......... .......... .......... ...
12     .......... .......... .......... ...
13     .......... .......... .......... ...
14     .......... .......... .......... ...
15     .......... .......... .......... ...
SALPAT .......... .......... .......... ...
```

a. Nucleotide analysis:

Both strains from Patient K presented 100% identity of the amino acid and nucleotide sequences (Annex Alignment 3). There were several silent point mutations observed in groups containing more than one test sample, including groups 1, 3, 8, 9, 11 and 15. In analyzing the strains from CSP VK210 group 1, using the sequence of BELPAT as the reference, we noted that proline (P) was coded by CCA and CCT, glutamine (Q) was coded by CAA and CAG, glycine (G) was coded by GGC, GGT and GGA, and aspartic acid was coded by GAC and GAT. The strain from patient A presented A/T transversions at nucleotide residues 111 and 273, C/T transitions at residues 201 and 33, and a C/A transversion at residue 387. The strain identified as "B" had a C/T substitution at nucleotide 201, a C/A nucleotide substitution at residue 333, and a C/A transversion at nucleotide 387. The strain isolated from patient "H" had one silent point mutation at nucleotide residue 459 (A/G). An A/G transition was found at nucleotide residue 135 in strains 136B, 126B-1 and 297A, and at residue 459 in both 297A and 294B strains.

Group 2 consisted of one test sample, identified by the letter G. In this amino acid sequence group, the codon for amino acid glycine (G) present in the beginning of each repeat block was polymorphic in the third position in repeat blocks 8 (A/T), 10 (T/C), 11 (C/A), 12 (A/C) and 13 (C/A) when compared to reference BELPAT. The codon for amino acid glutamine (Q) in the seventh amino acid position of the conserved repeat blocks was polymorphic in the third codon position in repeat blocks 8 (G/A), 9 (A/G), 13 (G/A), 14 (A/G) and 15 (A/G). Another polymorphism observed in repeat block 8 occurred in the codon for aspartic acid (D) in the second position of the block (C/T).

Seven non-silent mutations resulted in a change of the fifth amino acid on seven repeat blocks; all changes occurred in the second position in the codon. In repeat blocks 8, 11, 13 and 15, the amino acid aspartic acid (D) was replaced by alanine (A), as a result of the nucleotide transition of adenine (A) to cytosine (C). In repeat blocks 10, 12 and 14 the transition C/A occurred, changing amino acid alanine to aspartic acid.

In group 3, the nucleotide sequence in the strains isolated from patients C and 297B were identical. One silent mutation was found in the conserved region I of strain E; nucleotide guanine (G) at residue 48 was replaced by adenine (A). Group 4 presented GDRAAGQPA as the sequence of repeat block 20. Groups 5, 6 and 15 presented the motif characteristic of Salvador I strain (GDRAAGQAA) in the repeat block 20. There was one test sample, each in groups 4, 5 and 6 and two test samples in group 15, with identical nucleotide sequences. When compared to SALPAT, test sample 361A, representative of group 5, was polymorphic at the third position in amino acid residues 21 (A/T), 29 (A/T), 31 (C/T), 33 (A/T) and 54 (A.G).

Analysis of the nucleotide sequences from samples in groups 4, 5, 6 and 15 revealed a C/A polymorphism in the second position of codon numbers 115 (KA and 361A), 133 (KA only) and 151 (KA and 361A). This polymorphism resulted in the change of amino acid alanine (A) by aspartic acid (D) in the fifth position of repeat blocks 11, 13 and 15. An A/C polymorphism in codon numbers 97, 142 and 160 resulted in substitution of aspartic acid with alanine in the fifth position of repeat blocks 9, 14 and 16. This mutation only occurred in test sample KA. A non-silent G/C mutation occurred in the first position of codon 190 and 199 in repeat blocks 19, in test samples 66, 361A,

361B and 370B, and in repeat block 20, in test sample 66. This non-silent mutation resulted in the replacement of amino acid alanine (A) by a proline (P).

Test samples KA and KB presented identical nucleotide sequences and represented group 6 in the unique VK210 amino acid sequences found in the strains from Manaus. Compared to BELPAT, the codon for amino acid glycine (G) present in the beginning of each repeat block was polymorphic in the third position at repeat blocks 3 (T/A) and 4 (A/T). In addition, the second amino acid, aspartic acid (D), was polymorphic in the third position of the codon at repeat blocks 3 (T/C) and 6 (C/T). The codon for the amino acid glutamine (Q) was polymorphic in the third position of the codon at blocks 3 (A/G) and 4 (G/A). A non-silent (C/A) mutation in repeat block 5 resulted in the change of the fifth amino acid from aspartic acid to alanine. In repeat blocks 16, 18 and 19 a C/A substitution resulted in the replacement of proline (P) by alanine (A) in the eight position of the repeat block. In repeat block 20, there were three amino acid substitutions when test sample K was compared to BELPAT, these amino acids were also present in the SALPAT strain. The second amino acid, asparagine (N), was replaced by aspartic acid (D) when adenine was replaced by guanine in the first position of the codon. The third amino acid, glycine (G), was replaced by arginine (R) due to the replacement of codon GGT by AGA. The fifth amino acid, glycine (G), was replaced by alanine when a transversion (G/C) occurred in the second nucleotide in the codon. Repeat block 21 (GGNAGGQGQ) was identical to BELPAT, as was the rest of the nucleotide sequence.

In Group 7, represented by test sample, 246A, a number of silent mutations were noted. Glycine, the first amino acid in each repeat block, had polymorphisms in the third

position of the codons in blocks 3 (T/A), 4 (A/T), 6 (A/T), 10 (T/A) and 11 (C/T) when this strain was compared to BELPAT. Aspartic acid, the second amino acid of repeat blocks 3 (T/C), 6 (C/T), 14 (T/C), 15 (T/C), 16 (T/C) and 17 (T/C) also had silent mutations when compared to the reference strain. Another common variation was the transition between adenine and guanine in the codon for glutamine (Q) present in the seventh position of the repeat blocks 3 (A/G), 4 (G/A), 11 (G/A), 14 (A/G) and 15 (A/G). A non-silent mutation, changing the codon for alanine (GCT) by aspartic acid (GAT), was noted in the fifth position of repeat blocks 4, 9, 10, 12, 14, and 16. These codons were present in the same amino acid residue as the strain SALPAT, but test sample 246A presented the repeat block 20 GNGAGGQAA, characteristic of BELPAT.

In group 8, nucleotide sequences from test samples 827, 103A and 246B were identical, while the sequence from test sample 738 presented several silent mutations when compared to the other sequences in the group. Test sample 738 presented an A/T transversion at nucleotide residues 117 and 306, a C/T transition occurred in nucleotide residues 120, 390, 417, 444 and 471, a G/A transition was observed at residues 135 and 405, and a T/A transversion was present in residues 144 and 198. In group 9, strains presented 19 amino acid blocks; nucleotide sequences revealed that test samples 131, 136, 913, 370A were identical. Test sample 212 had a silent mutation for amino acid number 80; the third nucleotide adenine was replaced by thymidine. Group 10 was represented by a strain from patient 126A. This strain presented an extra amino acid insertion, alanine, prior to the 20 amino acid blocks that composed the repeat sequence. Group 10 also presented amino acids unique to these strains that were absent in the reference strains. A/T substitutions were noted in the third position of the condon for

glycine in the repeat blocks 1, 2, 4, 5 and 7; T/A occurred in blocks 9 and 10, C/A in block 11 and C/T in block 13. C/T polymorphism in the third position of the codon for aspartic acid was found in repeat blocks 1, 2, 6, 7 and 8; A/G polymorphism was observed in the third position of the codon for glutamine, in repeat blocks 9 and 15. An alanine by aspartic acid substitution occurred in the fifth position of repeat blocks 4, 9, 10, 12, 14, 16, 17 and 18 when a C/A transversion occurred in the second position of the codon. Some of these substitutions were also present in the SALPAT reference strain but others were unique to strain 126A. Block 19 was deleted and block 20 was that characteristic of the Belém strain, GNGAGGQAA.

Group 11, composed of test samples I and D, contained identical nucleotide sequences, presenting 19 repeats that were similar to the strains in group 9, with a difference in amino acid 67. Strains from group 11 presented an asparagine, encoded by AAT, while those in group 9 presented an aspartic acid encoded by GAT. Test sample 92A of group 12 and test sample 126B-2 of group 13, both presented 19 repeat blocks. A G/A silent mutation in the codon for glutamine (Q) was noted in residue 54. These two strains differed in amino acid residue 67. Strain 95A presented an asparagine (N) and 126B-2 an aspartic acid (D), which resulted from an A/G substitution in the first position of the codon.

Group 14 had one strain from test sample 95B; this strain had 20 repeat blocks. Comparison between this strain and both reference strains revealed substitutions on the amino acid at the fifth position of repeat blocks 12 (A/D), 13 (D/A), 14 (A/D) and 15 (D/A). Alanine (A) was coded by GCT and aspartic acid (D) was coded by GAT. There was a deletion of repeat block 19, and block 20 was identical to the Belém strain. When

95B was compared to BELPAT, silent mutations were noted at nucleotide residue 387 (C/A), 405 (G/A) and 459 (A/G).

b. Repeat Blocks Nucleotide Analysis:

The repeat block GDRAA/DGQPA, was the common repeat for blocks 1 to 19 among all of the sequences. Synonymous substitutions were more frequent in amino acids at positions 1, 2 and 7 of these repeats, and less frequent in amino acids 4, 6, 8 and 9. In the fifth amino acid position, only non-silent mutations were present, resulting in an alanine/aspartic acid polymorphism. Figure 28 summarizes these substitutions by amino acid position and frequency, according to the sequences from the primary infection. The extraordinary occurrence of the same A/D polymorphism at position five clearly demonstrates that the mutation has important relevance to the function of the parasite.

The frequency of alanine and aspartic acid among the first repetitive block of this region is presented by sequence group in Table 7. The number of alanines at the fifth position varied from zero to nine. This frequency distribution could indicate that there is a requirement for the parasite to retain aspartic acid at this position in at least half of the repeat blocks.

We then analyzed the frequency of alanine in the fifth position by patient treatment group (Figure 29). Among the admitted patients, nine had eight or nine alanines in the fifth position and two had none. Among the outpatients, eight had eight or nine alanines in the fifth position, and the rest had six or fewer, mostly three or fewer. These frequency distributions were not significantly different by Kruskal-Wallis test ($p=0.095$). This difference suggests that the presence of alanine at a high proportion, approximately 50%, of the fifth positions in the repeat blocks may be associated with pathogenicity.

Figure 28: Frequency of synonymous and non-synonymous substitution in the CSP repeat blocks 1 to 19.

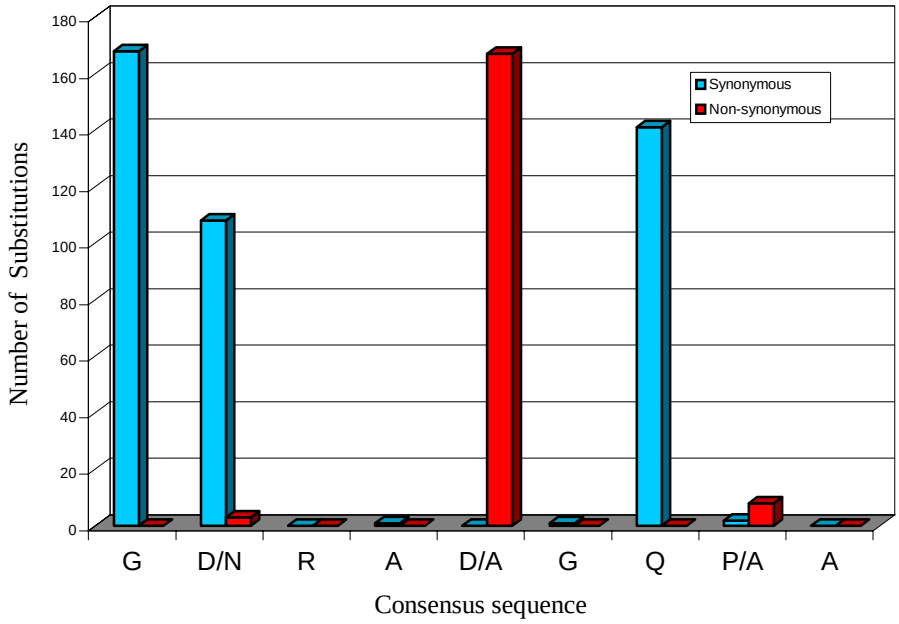


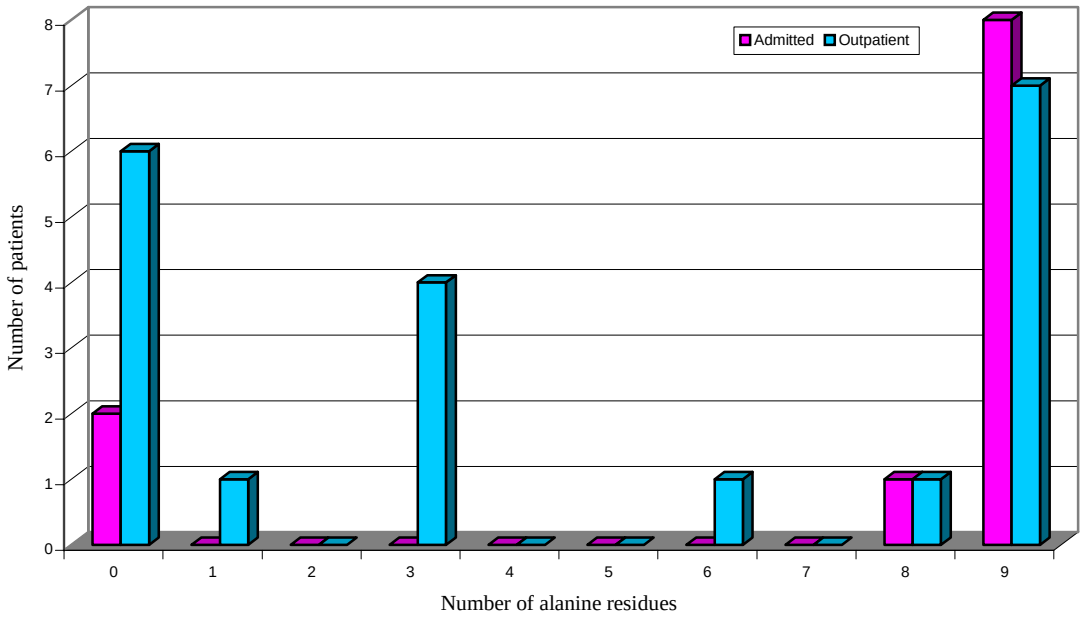
Table 7: Frequency distribution of alanine and aspartic acid in the fifth position of the repeat blocks 1 to 19, by amino acid sequence group.

Position 5 Amino Acid	CSP Amino Acid Sequence Group														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	9	8	9	8	6	9	3	3	0	0	0	1	1	8	8
D	10	10	9	11	13	10	16	15	17	18	17	17	17	10	11

We then considered the possibility that this A/D polymorphism was occurring as a result of biological selection. To test this possibility we evaluated the frequency of substitutions in sequences derived from paired samples from patients with second time point infections. These paired sequences are compared in Figure 26. Remarkably, we found 32 substitutions at this position among the pairs, and 29 involved D to A

substitutions while three involved the reverse. The strong tendency for evolution from D to A was consistent with the possibility that biological selection, perhaps immunological selection, occurred in these patients. The evidence supports an hypothesis regarding immunological selection regardless of whether the second time point infections were due to relapse, recrudescence, or reinfection, since it suggests that strains with high frequency of A at position five had selective advantage in any case. As discussed in detail below, we propose the hypothesis that alanine substitution at this position occurs as a result of escape mutation at a cytotoxic T cell epitope, and that strains with relatively high frequencies of alanine at this position may be associated with increased pathogenicity because of reduced susceptibility to immunological control.

Figure 29: Frequency of alanine residues in the fifth position of repeat blocks 1 to 19, distributed by patient treatment group.



c. Tree Search and Bootstrap Analysis:

Amino acid and nucleotide sequences were used to construct phylogenetic trees and to determine the relationship between the strains from Manaus and the reference strains Salvador I and Belém. The software programs PAUP 4.0b (Swofford, 2002) and MEGA 2.1 (Kumar et al., 2001) were used to search trees using neighbor-joining and heuristic search methods, using distance and parsimony criteria. Bootstrap analysis was used to determine the reliability of the estimate.

Amino acid alignment (Annex Alignment 2), from test samples and references Salvador I (SALPAT) and Belém (BELPAT) was used to construct the tree presented in Figure 30. The amino acid sequences in the alignment consisted of 282 characters; 282 of which were constant and 43 were variable and parsimony-informative. The tree is an unrooted, fifty percent majority rule consensus of 87 trees found by heuristic search under the optimality criterion of parsimony. Gaps in the amino acid alignment were treated as the 21st amino acid. Starting trees were obtained by stepwise addition and sequences were added randomly, with tree-bisection-reconnection serving as the branch-swapping algorithm. The bootstrap method with heuristic search was used to determine the reliability of the estimated groups. Bipartitions frequency of occurrence, bootstrap support values, for 1,000 replicates are indicated in the node of each branch, groups at relative frequency less than fifty percent are not shown in Figure 30. The distribution of the 15 CSP VK210 amino acid sequence groups of *P. vivax*, isolated from patients in Manaus, is represented in this tree. The phylogeny defined three main groups. One group (font in pink) was comprised of 15 sequences with amino acid sequences identical to the Belém strain, BELPAT (bootstrap support value of 80.7%). Another group (font in blue)

was comprised of sequences that were considered to be related to the Salvador I strain, SALPAT, based on the polymorphism at repeat block 20. The strain K was only weakly linked to the other members (bootstrap support value of 55.2%). There was significant support for the association of SALPAT with the strains 66, 370B and 361B (bootstrap support value of 82.9%). The inclusion of strain 361A was supported by a bootstrap value of 79.3%. These results indicate that, although strain K presented the SALPAT motif in repeat block 20, there was not strong statistical support for its relation to SALPAT. The third group (font in green) identified by the phylogenetic analysis was comprised of sequences with variable numbers of repeats and unique amino acids in the repeat blocks. The bootstrap support value was 59.5% for the entire group and 96.4% for the grouping of all sequences, excluding 246A. Sequence group 3, formed by strains C, E and 297B, had a support value of 98.5%. The introduction of the strains G (sequence group 2) and 95B (sequence group 14) to this group occurred 79.4% of the time.

During the bootstrapping procedure, strains 95A (sequence group 12) and 126B-2 (sequence group 13) were grouped together 100% of the time. These sequences presented 19 repeat blocks and only differed by one N/D polymorphism in amino acid 67. Strains D and I of sequence group 11 grouped 100% of the time with the strains 131, 136, 212, 913 and 370A of sequence group 8. Amino acid groups 11 and 8 each presented 19 repeat blocks and differed only by a N/D polymorphism in amino acid 67.

Distance criterion and neighbor-joining were used to infer the phylogeny of the CSP gene. The amino acid sequence alignment revealed a similar topology (Annex Figure 1), based on similarities and divergences to the reference strains. Strains related to BELPAT were contained in one group while strains related to SALPAT divided in two

different groups, one containing strain K and another composed of 361A, 66, 370B, 361B and SALPAT. The strains presenting unique amino acid sequences were divided in two main groups: one composed of strains G, 95B, 297B, C and E and another composed of the remaining strains.

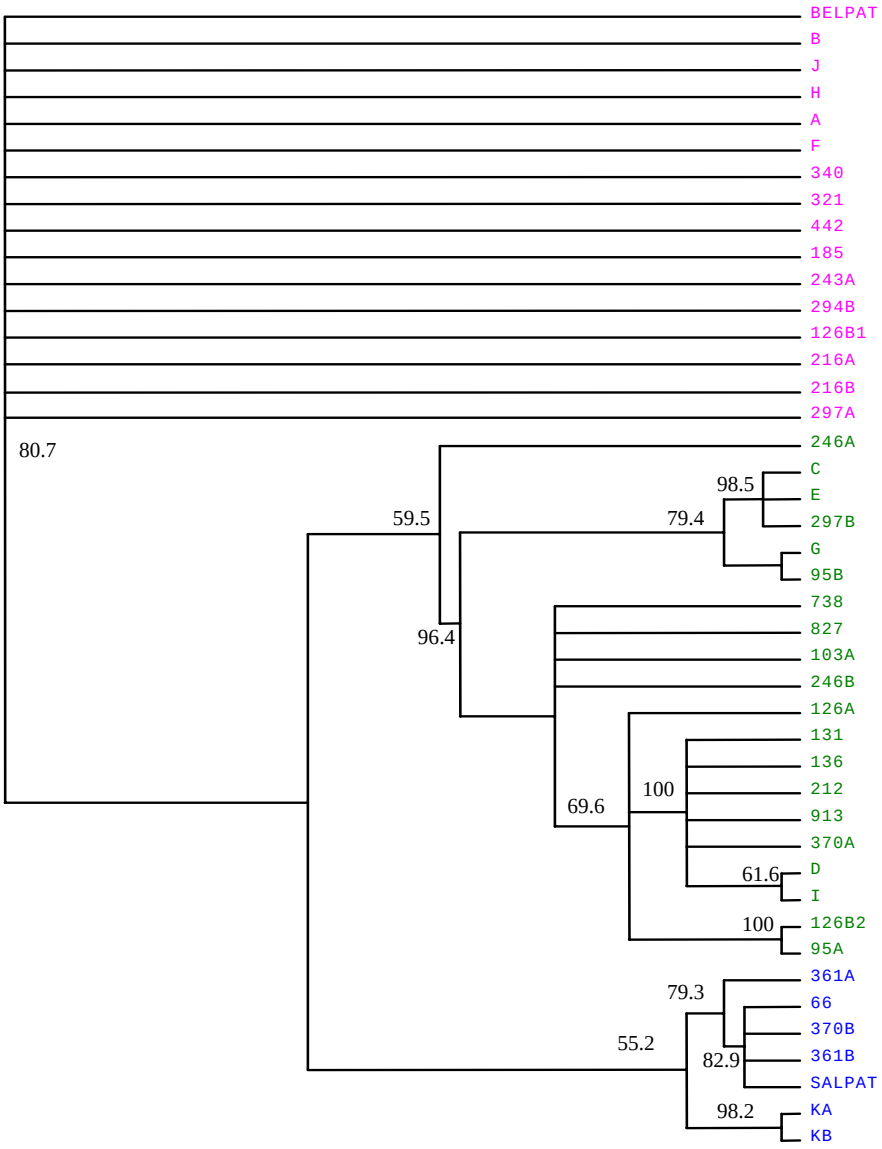
Nucleotide sequence alignment was also used to infer the phylogeny of the CSP gene using both the heuristic search under the parsimony (Annex Figure 2) and neighbor joining criteria (Annex Figure 3). The fifty percent majority rule consensus of the trees, found by heuristic search, revealed a separation of the fifteen groups found in the amino acid sequence analysis. Test sample 738, separate from the other strains in sequence group 8 appeared proximal to strains G (sequence group 1) and 95B (sequence group 14). This finding was due to the variation noted in the nucleotide sequence of test sample 738 when compared to 827, 103A and 246B, all presenting sequence group 8, but with the same nucleotide sequence.

The distance-based approach also identified strain 738 as a neighbor of strains 95B and G. Other small variations noted in the topology of the neighbor-joining tree reflected the point mutations present in the strains from amino acid sequence group 1. Strains A and B were located in a branch neighbor to the main amino acid sequence group 1 and the strains H and 294B were presented as a sub-set in the main group. This variation was due to the presence of silent mutations in the nucleotide sequence of the strains in group 1.

Despite the variation observed when using different algorithms for analysis and for alignments based on nucleotide sequences, it was demonstrated that three main sequence groups were present in the strains from Manaus. One group was identical to the

Belém strain, BELPAT, while the second group of strains was Salvador I-like and the third group of strains was considered Belém-like. The strains that were classified as Belém-like and Salvador I-like presented unique characteristics that resulted in the subdivision of these groups.

Figure 30: Fifty percent majority rule consensus of trees found by heuristic search showing inferred phylogeny of CSP amino acid sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELPAT). Numbers in nodes are bootstrap support values (cutoff value of 50%).



Comparison of Sequences of Study Strains to Sequence Available in Gene Bank:

Sequences corresponding to the same segment of the CSP gene were retrieved from Gene Bank (Table 8). Nucleotide and deduced amino acid sequences were aligned with sequences of strains from Manaus with gaps adjusted manually (Annex Alignments 4 and 5). PAUP 4.0b10 (Swofford, 2002) and MEGA 2.1 (Kumar et al. 2001) were used to find trees based on distance and parsimony criteria and to calculate the bootstrap support values of the topologies found.

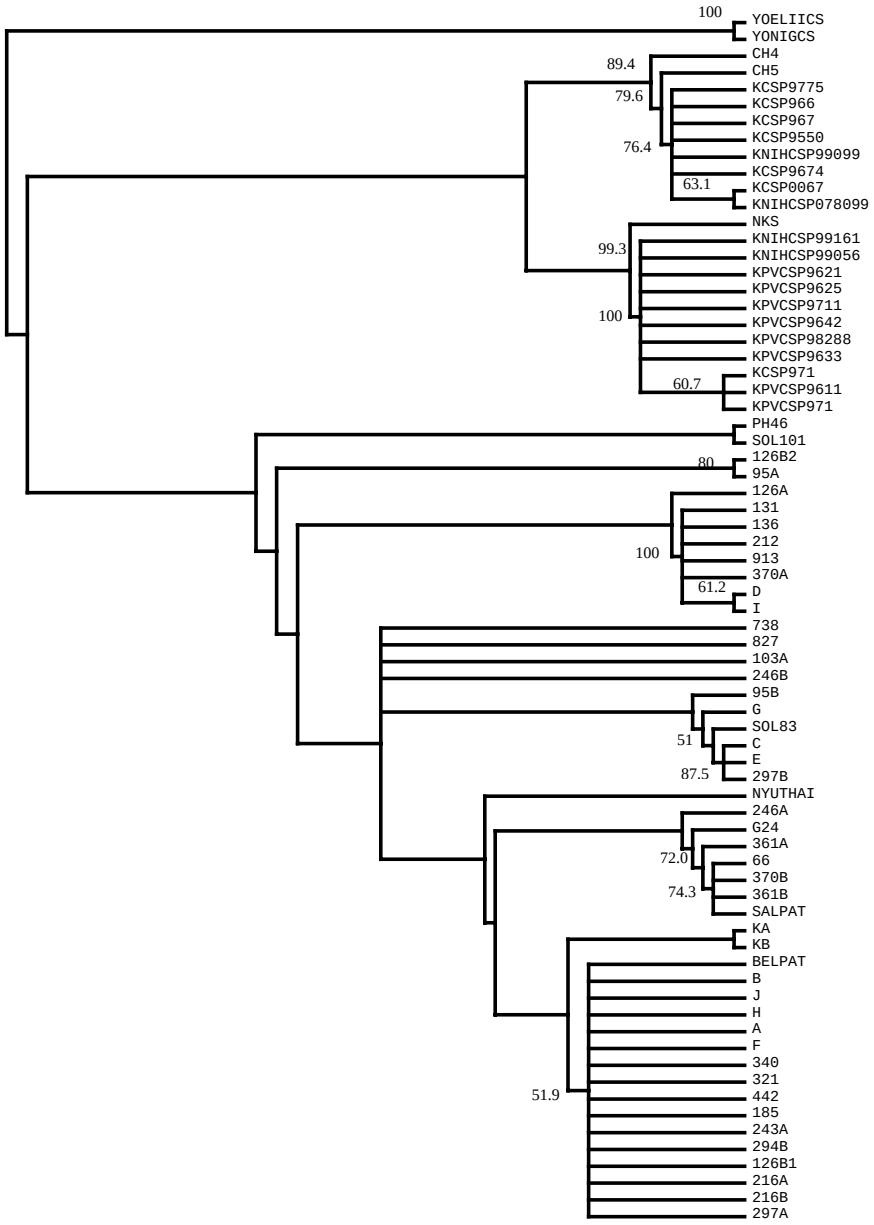
Alignment data revealed unique characteristics present in the sequences from Korea. Repeat GNGAGGQP/AA was noted in various repeat blocks in the sequence of the strains. The insertion of the partial repeats GGNA and ANKKAEDA were present in the post-repeat area.

A consensus of 100 trees found using a heuristic search under the optimality criterion of parsimony is presented in Figure 31. The amino acid alignment consisted of 302 characters, of which 53 were constant and 249 variables. Of the variable characters, 62 were parsimony non-informative and 187 were parsimony informative. Starting trees were obtained by stepwise addition, sequences were added at random and tree-bisection-reconnection was the branch-swapping algorithm. The bootstrap method with heuristic search was used to determine the reliability of the estimated groups. Bipartitions frequency of occurrence, bootstrap support values, for 1,000 replicates are indicated in the node of each branch. Groups at relative frequency of less than fifty percent are not shown in the tree.

Table 8: CSP gene sequences obtained from Gene Bank.

Access Number	Isolate	Year	Country	Reference
Salvador I and Belém Strains				
J02751	Sal – I	1987	El Salvador: La Paz	McCutchan et al. 1985
M11926	Belém	1988	Brazil	Arnot et al. 1985
Outgroup sequence				
J02695	*Plasmodium yoelii* L	1987	Rodent malaria	Lal et al., 1987
M58295	*P. yoelii nigeriensis*	1990	Rodent malaria	Colomer-Gould & Enea, 1990
AF164603	KPVCSP96-11	1999	South Korea	Lim et al. 2001
AF164604	KCSP95-50	1999	South Korea	Lim et al. 2001
AF164605	KPVCSP97-1	1999	South Korea	Lim et al. 2001
AF164605	KCSP97-1	1999	South Korea	Lim et al. 2001
AF164606	KCSP97-75	1999	South Korea	Lim et al. 2001
AF164607	KCSP96-6	1999	South Korea	Lim et al. 2001
AF164608	KCSP96-74	1999	South Korea	Lim et al. 2001
AF240459	South Korea – SK	2000	South Korea	Sohn et al. 2000
AF316579	KNIHCSP99-056	2000	South Korea	Lee et al. 2000
AF316581	KNIHCSP078-099	2000	South Korea	Lee et al. 2000
AF316582	KNIHCSP99-099	2000	South Korea	Lee et al. 2000
AF316583	KNIHCSP99-161	2000	South Korea	Lee et al. 2000
AF436890	KCSP96-7	2001	South Korea	Lim et al. 2001
AF436891	KCSP00-67	2000	South Korea	Lim et al. 2001
AJ278611	KPVCSP96-25	2000	South Korea: Yonchon	Lim et al. 2001
AJ278612	KPVCSP96-42	2000	South Korea: Pajoo	Lim et al. 2001
AJ292977	KPVCSP98-288	2000	South Korea: Pajoo	Lim et al. 2001
AJ295636	KPVCSP97-11	2000	South Korea: Pajoo	Lim et al. 2001
AJ297403	KPVCSP96-33	2000	South Korea: Pajoo	Lim et al. 2001
AJ400910	KPVCSP96-21	1996	South Korea: Pajoo	Lim et al. 2001
M20670	North Korean Strain – NKS	1988	North Korea	Arnot et al. 1988
M34697	NYUTHai	1990	Thailand	Arnot et al. 1990
U08978	CH-4, CH-6, CH-7	1994	P.R. China	Mann et al. 1994
U08979	CH-5	1994	P.R. China	Mann et al. 1994
U08980	PH-46, PH-52, PH-54, PH-63, PH-81, PH-84	1994	Philippines	Mann et al. 1994
U08981	PH-79	1994	Philippines	Mann et al. 1994
U08982	Sol-83	1994	Solomon Islands	Mann et al. 1994
U08983	Sol-101	1994	Solomon Islands	Mann et al. 1994
U09737	Gabon – G24	1994	Gabon	Qari et al. 1994

Figure 31: Fifty percent majority rule consensus of trees found by heuristic search showing inferred phylogeny of CSP amino acid sequence of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELPAT), and sequences from Gene Bank. Tree rooted with the outgroup *P. yoelii*. Numbers in nodes are Bootstrap support values (cutoff value of 50%).



All strains from Korea and China formed a group separate from the strains from Manaus (Brazil), Philippines, Solomon Islands and Thailand. The strains from Manaus formed groups of similar topology. A main group composed of strains with an amino acid sequence identical to BELPAT. Another group was composed of strains related to the strain Salvador I and a second group of strains with unique amino acid sequence related to the Belém strain.

The strain from Solomon Islands, SOL83, was weakly associated with the strains presenting as sequence group 3 (C, E and 297B). When these four sequences were compared, we noted four polymorphic sites within the repeat blocks. There was a G/A amino acid polymorphism at residue 21, a deletion of amino acid 29 in SOL83, a D/A polymorphism at position 160, and an A/D polymorphism at residue 169. The strain SOL83 presented an extra incomplete repeat, GGNA, prior to the last repeat block of the amino acid sequence.

The alanine/aspartic acid polymorphism in the fifth position of the common repeat block GDRAA/DGQPA was also noted among the strains from Gene Bank. Interestingly, the strain from Thailand, NYUTHAI, presented the deletion of nine amino acids in residues 52, 97, 106, 124, 142, 160, 169, 178 and 187. All of these deletions represented the loss of amino acid number five from the repeat block. This strain did not form a significant group with the other strains. The strain from Gabon, G24, had a threonine (T) at the position immediately before the first repeat block. Differences were noted in seven amino acid residues when G24 was compared to strain 246A and in nine residues when compared to 361A. The association of Gabon 24 with strains 246A and 361A was not supported by a significant bootstrap value.

Nucleotide sequences were aligned and to infer the phylogeny of CSP gene. A total of 908 characters were present in the alignment, 296 of which were constant and 612 were variable. Within the variable characters, 369 were not parsimony informative and 243 were parsimony informative. During a heuristic search under the parsimony criterion, gaps were considered a fifth base (Annex Figure 4). The grouping was similar to the one described for the amino acid sequences, with few variations of significance. NYUTHAY was in the same group as the Belém strain with a bootstrap support value of 60.1%.

Strain 738 was no longer in the same branch as other strains from the sequence group 8. It was associated with strains G and 95B, with a bootstrap support value of 60.4%. This same change occurred when strains from Manaus were analyzed without the introduction of foreign strains, and was explained by the various silent mutations present in the nucleotide sequence of test sample 738.

Clones Isolated from Test Sample C:

DNA extracted from test sample C yielded a mixed infection, as depicted by double band in the agarose gel. Both bands were cloned, identifying a mixed infection of *Plasmodium vivax* CSP types. Three clones were identified as VK210. Clone C and C14 had identical amino acid sequences and differed from Clone C18 in two residues. Two clones, C10 and C20, were identified as *P. vivax*-like and differed in two amino acid residues (Figure 32).

Figure 32: Alignment of deduced amino acid sequences of CSP gene segments from the clones isolated from study sample C.

VK210 clones:

```
C   KLKQPA-GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA
C14 ......-......... ......... ......... ......... ......... .........
C18 ......-......... ......... ......... ......... ......... .........

C   GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA
C14 ......... ......... ......... ......... ......... .........
C18 ......... ......... ......... ......... ...T..... .........

C   GDRAAGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRAAGQPA
C14 ......... ......... ......... ......... ......... .........
C18 ......... ......... .....R... ......... ......... .........

C   GNGAGGQAA GGNAGGQGQ NNEGANAPNEKSVKEYLDKVRAT VGTEWTPCSV
C14......... ......... ...................... ..........
C18......... ......... ...................... ..........

C   TCGVGVRVRR RV
C14 .......... ..
C18 .......... ..
```

vivax-like clones:

```
C10 KLKQP VPGANQEGGAA APGANQEGGAA APGANQEGGAA APGANQEGGAA APGANQEGGAA
C20 ..... ........... ........... ........... ........... ...........

C10 APGANQEGGAA APGANQEGGAA APGANQEGGAA APGANQEGGAA APGANQEGGVA
C20 ........... ........... ........... ........... .........A.

C10 APGANQEGGAA APGANQEGGAA APGANQEGGAA APGANQGGGAA APGANRGGGAA
C20 ........... ........... ........... ........... .....Q.....

C10 APGANQGGGAA APGANQEGGAA APGANQGGAKS AGGQGQ NNEGANKPDEKHVKEYLEKIRST
C20 ........... ........... ........... ...... .......................

C10 VGTEWTPCSV TCGKGVRVRR KL [254]
C20 .......... .......... .. [254]
```

3.2.5 MSP-1 gene:

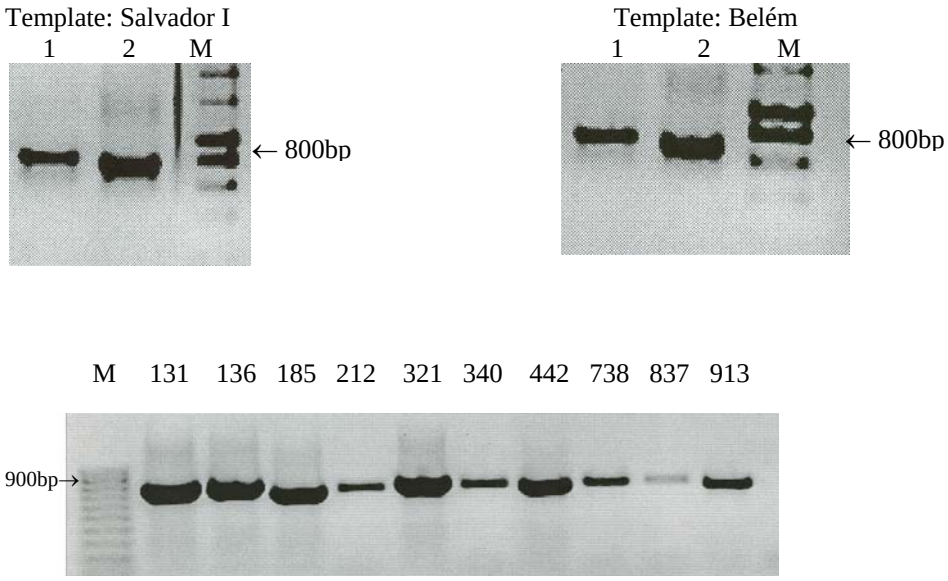
3.2.5.1 Standardization and Use of the PCR to Amplify the Regions between Interspecies Conserved Blocks (ICBs) 5 and 6 of the MSP-1 gene of *P. vivax*:

To amplify the region between ICBs 5 and 6 of *P. vivax* MSP-1, we designed two additional primers to complement those described by Putaporntip and colleagues (1997). Primers were used in a nested PCR reaction (PV1/PV2 followed by 5F1/6R1) or in a semi-nested reaction (PV1/PV2 followed by 5F1/PV2). The final protocol was successfully standardized (Figure 33).

Both *P. vivax* reference strains Salvador-1 and Belém MSP-1 genes were amplified by the reactions in the protocol. Size variation observed in the electrophoretic images of the PCR products was related to the insertion and deletions observed in the DNA sequence of the alleles present in each strain (Figure 33). There was no evidence of mixed infections in the samples included in this study, as there were no double bands in the electrophoreses gel or double peaks in the sequence histograms.

There were 32 patients included in the study, eleven treated in the hospital and 22 treated as outpatients in the ambulatory clinic. Ten patients in the outpatient group and one in the hospitalized group presented at second time-points of infection during treatment for malaria. MSP-1 gene from seven outpatients and one hospitalized case were amplified by PCR and sequenced. PCR products from all but one test sample, 361A, were directly sequenced.

Figure 33: Standardization and application of the PCR protocol to amplify *P. vivax* MSP-1 gene between ICBs 5 and 6. A: Standardization using reference strains Salvador I and Belém, reaction 1 used primer pair 5F1/PV2 and reaction 2 used primer pair 5F1/6R1. B: PCR products obtained from study samples using primer pairs PV1/2 and 5F1/6R1, for the first and second reactions, respectively. Lanes M contained 100 bp markers. Remaining lanes contained DNA amplified from study samples, as indicated. Electrophoreses were performed on 1.5% agarose gel in tris acetate - EDTA buffer and stained with ethidium bromide.



3.2.5.2 Phylogenetic Analysis of MSP-1 gene Sequences:

The PCR products were purified and sequenced using forward and reverse primers. Forward and reverse sequences were edited, assembled in contigs, and aligned using Megalign 3.06b (DNASTAR Inc.) and Clustal X 1.83. Gaps were edited manually to avoid the separation of nucleotides from the codons. DNA sequences were translated to amino acid using EditSeq 3.88 (DNASTAR Inc.). Amino acid alignments were used to classify the *P. vivax* MSP-1 sequences based on comparison with the reference strains Salvador I and Belém.

To study the phylogeny of *P. vivax* based on the MSP-1 gene, nucleotide and amino acid sequence alignments were prepared by combining sequence data from the samples, reference strains Salvador I and Belém, and Gene Bank sequences. Analyses were performed with and without the out-group root, *P. yoelii*.

Strains from Manaus and References Salvador I and Belém:

The phylogeny of *P. vivax* from the Amazonas State, based on the variable region between ICBs 5 and 6 on the MSP-1 gene, was inferred using information from patients' sequences, reference strains Salvador I and Belém and Gene Bank sequences. The MSP-1 sequence of *P. yoelii* was used as the out-group root in some of the analyses.

Dr. Barnwell of the CDC provided genomic DNA from Salvador I and Belém strains. PCR product obtained using this genomic DNA was sequenced and compared to Gene Bank sequences. The sequence of the Salvador I strain (SALPAT) DNA obtained from Dr. Barnwell was 100% identical to the Gene Bank Salvador I sequences AF435593 and M75674. The Belém strain (BELPAT) was identical to sequence AF435594 but

differed from sequence M60807 (BELEM) by an A ↔ T inversion at positions 164 ↔ 165.

A comparison between Salvador I and Belém strains revealed a conserved region (CR) surrounded by variable regions within the region of interest. The CR extended from residues 152 to 173 in the alignment in Figure 34. In the variable region between conserved block 5 (CB5) and CR, Belém and SALPAT differed at 81/132 residues. Within this variable region, Belém contained a 23-residue polyglutamine sequence not seen in SALPAT. Carboxyl to the CR was a variable region, 26 amino acids in length, nine of which differed between the two reference strains.

An examination of these sequences revealed that variability among them reflected recombination events. Sequences shown in Figure 34, numbered 1, 2, and 3, differed from the Belém sequence mostly with respect to the length of the polyglutamine sequences. Sequence number 4 was similar to sequences 1, 2, and 3, except in the carboxy terminal variable segments, identified in the figure as recombination segment IV (RSIV), which was clearly SALPAT-like. This result indicated that sequence 3 had resulted from recombination occurring between Belém and Salvador I-like progenitors, at a position at or near the CR. Sequences 10, 12, and 13 were both SALPAT-like throughout, while sequences 5 to 9 displayed evidence of recombination.

Sequences 5, 6, and 7 were SALPAT-like in the segment corresponding to the first 37 amino acids of the first variable region, and identified as RSI in Figure 34. The remainders of sequences 5, 6, and 7 corresponded to Belém sequences. Sequences 8 and 9 consisted of a SALPAT-like sequence in RSI and RSIV, and were otherwise Belém-like. Sequence 13 was SALPAT-like throughout, except for four amino acids,

polyglutamine sequence, identified as RSII in Figure 34. These data supported the interpretation that the sequences were derived from Belém and Salvador I-like progenitors, with recombinatorial events occurring at the termini of RSII and near CR. Other than variations resulting from recombination, there was minimal sequence variation observed. The distribution of all strains by amino acid sequence group is presented in Table 9.

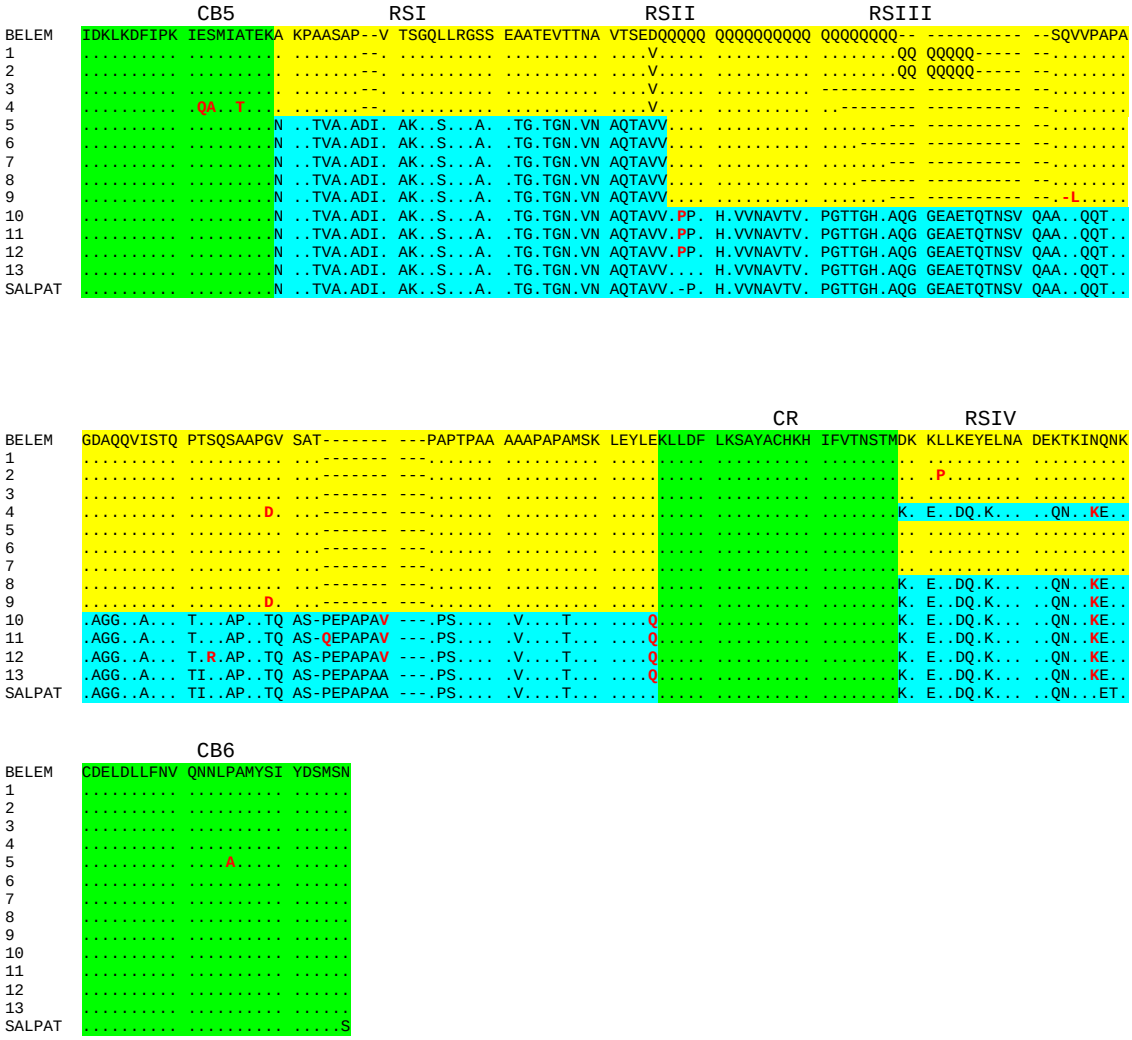
Table 9: Classification of the amino acid sequences from Manaus strains according to MSP-1 sequence and patient treatment group.

Sequence groups	Patient Treatment Group		
	No relapse	Relapse*	Admitted
1		126A	
2		361A	
3	913		H
4		297A, 294A	G
5		103A, 246A	
6	66, 212		A, J
7	738, 827	370A	C, KA
8			E
9	185, 340, 442	95A, 243A	
10	321	216A	F
11		216B**	
12			D
13	131, 136		B, I

* First time-infection sample represented

** Only amino acid from the second time-point infection that presented a different amino acid sequence

Figure 34: Deduced amino acid sequences of MSP-1 gene segments from study samples and references Belém (M60807) and Salvador I (SALPAT). Belém was used as the reference for the alignment. Amino acids identical to the sequence are represented by dots, unique amino acids are shown in red, and amino acids identical to the Salvador I strain are shown in bold. Regions are identified as conserved blocks 5 and 6 (CB6 and CB6), recombination segments (RS) I-IV, and conserved region (CR).

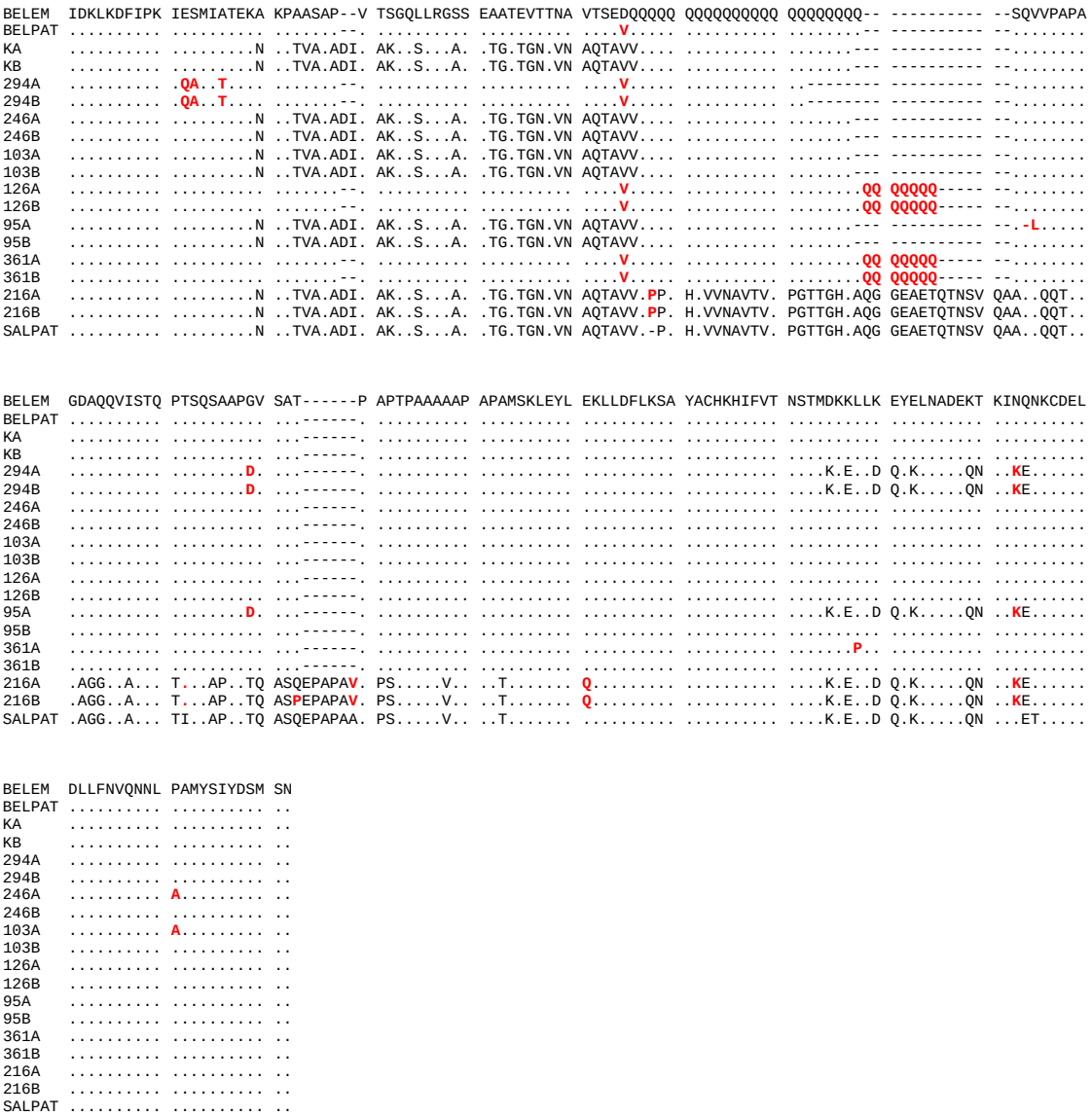


Strains from Second Time-Point Infection from Patients Treated as Outpatients:

In the samples selected for this study, 21 originated from patients treated as outpatients in the Malaria clinic of the FMT-Am in Manaus, Amazonas. In ten of these patients, malaria parasites were detected at second-time points during treatment. Seven of these ten patients had MSP-1 genes from the second time-point PCR-amplified and sequenced using forward and reverse primers. One patient (test sample K) in the group admitted for treatment in the hospital, also had a second time-point infection, from which MSP-1 gene was PCR-amplified and sequenced.

Forward and reverse sequences were edited, combined in contigs, and translated to amino acids using DNASTAR software packages. The aligned amino acid sequences of these genes are presented in Figure 35. Early and late sequences obtained from patients K, 126 and 294 presented the same amino acid sequences. A single amino acid substitution distinguished samples 103A from 103B, 216A from 216B, 246A from 246B, and 361A from 361B. Samples 95A and 95B differed due to the recombinatorial exchange of RSIV. The amino acid sequence presented by 361B was identical to the amino acid sequence of MSP-1 group 1. The amino acid sequence from strains 95B, 103B, and 246B were identical to those of MSP-1 group 7.

Figure 35: MSP-1 amino acid sequence alignment of strains from patients that presented two time-point infections during treatment for *P. vivax* infection. Belém was used as the reference strain for the alignment. Identical amino acids are presented by dots, gaps are presented by dashes, and amino acids unique to strains from Manaus in red.



Strains from Manaus: Strains Isolated from the Second Time-Point Infection.

There was limited variation in the nucleotide sequences of strains within each amino acid sequence group and substitutions were consistently dimorphic. There was no variation in the nucleotide sequence of strains from amino acid groups 1, 3, 4, 6, 9, and 13. There was one nucleotide difference between strains 103A and 246A of amino acid sequence group 5. In the amino acid sequence group 10, the strains 321 and 216 A presented the same nucleotide sequence, as they both differed from strain F by one nucleotide difference. Strains in the amino acid sequence group 7 presented two sets of nucleotide sequences. Strains 738, KA, KB and 95B were identical and differed from 827, 103B, 246B, 370B, and C.

Tree Search and Bootstrap Analysis:

Amino acid and nucleotide sequence alignments were used to construct phylogenetic trees and further examine the relationships between the sample sequences and references Salvador I and Belém. The software packages PAUP 4.0b (Swofford, 2002) and MEGA 2.1 (Kumar e al., 2001) were used to infer the phylogeny of the strains using distance and parsimony approaches. Bootstrap analysis was used to evaluate the robustness of the groups formed by neighbor-joining and heuristic search.

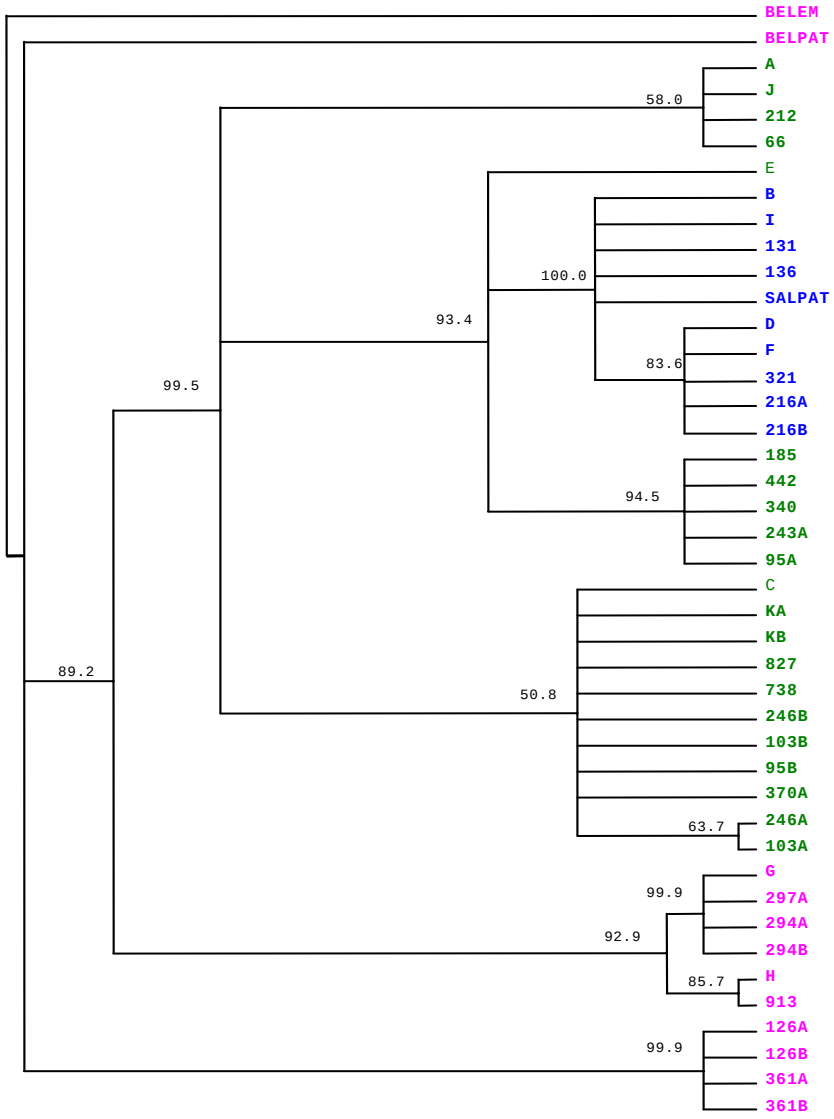
A fifty percent majority rule consensus of 162 trees found by Heuristic search under the optimality criterion of parsimony is presented in Figure 36. The amino acid alignment consisted of 222 total characters, 120 of which were constant, and 102 were variable. Of the 102 variable characters, four were parsimony non-informative and 98 were parsimony informative. Gaps were treated as the 21st amino acid. Starting trees were obtained by stepwise addition, sequences were added randomly and tree-bisection-

reconnection was the branch-swapping algorithm. The bootstrap method with heuristic search was used to determine the reliability of the estimated groups. Bipartitions frequency of occurrence, bootstrap support values, for 1,000 replicates were indicated in the node of each branch. Groups at relative frequency of less than fifty percent have been omitted. Test samples are color-coded based on the main amino acid sequence groups, pink for Belém-like, Blue for Salvador I-like and green for hybrids.

Results from the phylogenetic analysis were consistent with the observation that recombination was a major source of variation among the sequences. In the analysis shown in Figure 36, Belém-like sequences were separated from the other Salvador I-like and hybrid sequences. A bootstrap value of 99.5% isolated the Belém-like sequence groups from the others, Salvador I-like and hybrids. In the Belém-like group, variation in the number of polyglutamine residues and recombination at RSIV accounted for a significant separation of three subgroups, all presented significant bootstrap support value.

A bootstrap support value of 93.4% supported the separation of Salvador I-like strains and the hybrid groups with amino acid sequence 8 and 9 from the other strains. The Salvador I group separated from the hybrids 100% of the time. The separation of strains from amino acid group 9 occurred 94.5% of the time. The other hybrid groups did not have a significant bootstrap support value supporting their separation.

Figure 36: Fifty percent majority rule consensus of trees found by heuristic search showing inferred phylogeny of MSP-1 amino acid sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT). Numbers in node are Bootstrap support values (cutoff value of 50%).



Distance analysis of the amino acid alignment (Annex Figure 5) yielded similar topology and emphasized the differences among amino acid sequences. The hybrid groups were separated based on the recombination events at the carboxy terminal, RSIV. Nucleotide alignment consisted of 668 total characters, 439 of which were constant, and 229 were variable. Of the variable characters, seven were parsimony non-informative and 222 were parsimony informative. This alignment was used to build trees using heuristic search under parsimony and neighbor-joining (Annexed Figures 6 and 7). Synonymous substitutions within MSP-1 groups influenced the topology.

Comparison between Sequences from Amazonas and Gene Bank:

P. vivax MSP-1 nucleotide sequences corresponding to sample segments were retrieved from the Gene Bank (Table 10). Nucleotide and deduced amino acid sequences were aligned with strains from Manaus, with gaps adjusted manually (Annex Alignments 8 and 9). Distance and parsimony-based approaches were used to search for trees, and bootstrap analysis was used to determine the robustness of the groups formed.

Recombination events were the major source of sequence variation, as observed in the analysis of the Manaus strains. Nine of the thirteen amino acid sequence groups found in our study were determined to be unique when compared to the sequences from Gene Bank. The unique strains were found in groups 1, 2, 4, 5, 7, 8, 9, 11, and, 12. Amino acid sequences from strains in groups 3, 10, and, 13 were identical to clones from Brazil, BR07, BP13, BP63, and, BP30, respectively. Strains in group 6 had amino acid sequences identical to strains from Thailand, TD29, TD403, TD425B, and, TD458B. Figure 37 presents the unique amino acid sequences found among strains from Manaus and Gene Bank.

A bootstrap 50% majority-rule consensus tree is presented in Figure 38. Heuristic search with optimality criterion set to parsimony was used, starting trees were obtained by stepwise addition. The sequences were added randomly and the tree-bisection-reconnection was the branch-swapping algorithm. The bootstrap method with heuristic search was used to determine the reliability of the estimated groups. Bipartitions frequency of occurrence, bootstrap support values, for 1,000 replicates are indicated in the node of each branch.

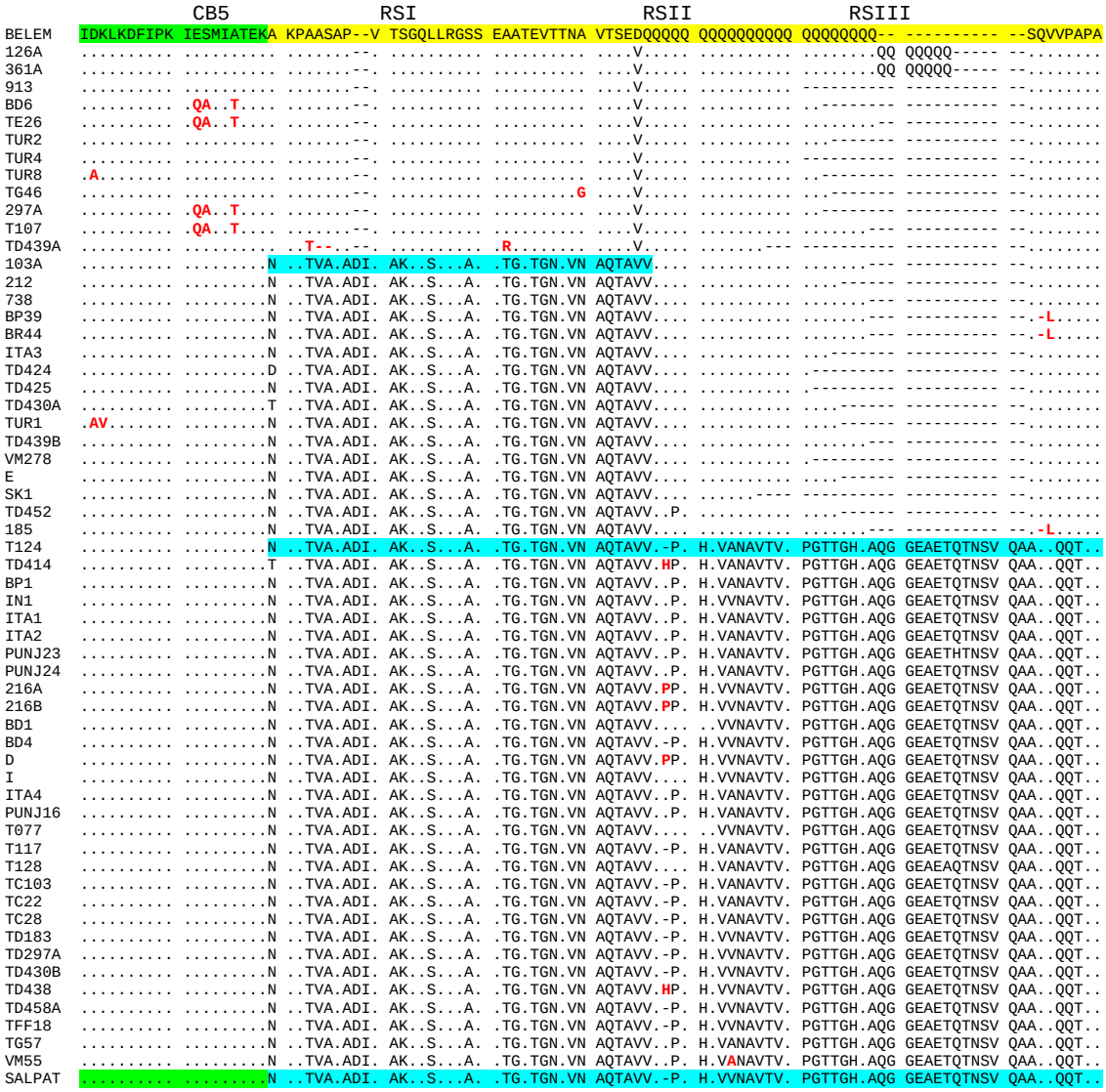
As observed in the strains from Manaus, recombination events were a major source of variation among strains from Gene Bank. In Figure 38, two main groups were generated. One consisted of strains with the Salvador I-like sequence, and was supported by a bootstrap value of 100%. Another group, formed by Belém-like and recombinant sequences was not supported by bootstrap. The separation of the Belém-like sequences from the hybrids was statistically significant (99.7%).

Table 10: Sequences from Gene Bank used as reference for the MSP-1 gene

Access#	Isolate	Year	Country of Origin	Reference
Out group				
J04668	*P. Yoelii* - YM – YOELII	1990	Rodent malaria	Lewis, 1990
Reference sequences:				
AF435593	SALVADOR (Sal 1)	2001	Brazil	Putaporntip et al., 2002
AF435594	Belem	2001	Brazil	Putaporntip et al., 2002
M60807	BEL91 (Belem)	1991	Brazil	Del Portillo et al., 1991
M75674	Sal (Sal 1/PV200)	1992	El Salvador	Gibson et al. 1992
AF435595	T064	2001	Thailand	Putaporntip et al. 2002
AF435596	T077	2001	Thailand	Putaporntip et al. 2002
AF435597	T107	2001	Thailand	Putaporntip et al. 2002
AF435598	T124	2001	Thailand	Putaporntip et al. 2002
AF435599	T131	2001	Thailand	Putapornitp et al. 2002
AF435601	TC22	2001	Thailand	Putaporntip et al. 2002
AF435602	TC28	2001	Thailand	Putaporntip et al. 2002
AF435603	TC103	2001	Thailand	Putaporntip et al. 2002
AF435604	TD29	2001	Thailand	Putaporntip et al. 2002
AF435605	TE26	2001	Thailand	Putaporntip et al. 2002
AF435606	TF14	2001	Thailand	Putaporntip et al. 2002
AF435607	TF127	2001	Thailand	Putaporntip et al. 2002
AF435608	TFF18	2001	Thailand	Putaporntip et al. 2002
AF435609	TG40	2001	Thailand	Putapornitp et al. 2002
AF435610	TG44	2001	Thailand	Putaporntip et al. 2002
AF435611	TG46	2001	Thailand	Putaporntip et al. 2002
AF435612	TG48	2001	Thailand	Putaporntip et al. 2002
AF435613	TG55	2001	Thailand	Putaporntip et al. 2002
AF435614	TG57	2001	Thailand	Putaporntip et al. 2002
AF435615	TV400	2001	Thailand	Putaporntip et al. 2002
AF435616	BD1	2001	India: Bangladesh	Putaporntip et al. 2002
AF435617	BD2	2001	India: Bangladesh	Putaporntip et al. 2002
AF435618	BD4	2001	India: Bangladesh	Putaporntip et al. 2002
AF435619	BD6	2001	India: Bangladesh	Putaporntip et al. 2002
AF435620	BD9	2001	India: Bangladesh	Putaporntip et al. 2002
AF435622	BP1	2001	Brazil	Putaporntip et al. 2002
AF435623	BP13	2001	Brazil	Putaporntip et al. 2002
AF435624	BP29	2001	Brazil	Putaporntip et al. 2002
AF435625	BP30	2001	Brazil	Putaporntip et al. 2002
AF435627	BP39	2001	Brazil	Putaporntip et al. 2002
AF435629	BP63	2001	Brazil	Putaporntip et al. 2002
AF435630	BR07	2001	Brazil	Putaporntip et al. 2002
AF435631	BR44	2001	Brazil	Putaporntip et al. 2002
AF435632	VM55	2001	Vanuatu	Putaporntip et al. 2002
AF435634	VM278	2001	Vanuatu	Putaporntip et al. 2002
AF435635	SK1	2001	South Korea	Putaporntip et al. 2002
AF435636	SK2	2001	South Korea	Putaporntip et al. 2002
AF435637	SK3	2001	South Korea	Putaporntip et al. 2002
AF435638	SK4	2001	South Korea	Putaporntip et al. 2002
AF435639	IN1	2001	India	Putaporntip et al. 2002
AJ250399	ThaiBK	1999	Thailand	Severnini et al. 2002

AJ250728	ITA2	1999	Italy Imp.from:India	Severnini et al. 2002
AJ250729	ITA1	1999	Italy Imp.from:India	Severnini et al. 2002
AJ494826	TUR1	2002	Turkey: Adana, Cukurova plain	Severnini et al. 2002
AJ494827	TUR2	2002	Turkey: Adana	Severnini et al. 2002
AJ494828	TUR3	2002	Turkey: Adana	Severnini et al. 2002
AJ494829	TUR4	2002	Turkey: Adana	Severnini et al. 2002
AJ494830	TUR5	2002	Turkey: Adana	Severnini et al. 2002
AJ494831	TUR7	2002	Turkey: Adana	Severnini et al. 2002
AJ494832	TUR8	2002	Turkey: Adana	Severnini et al. 2002
AJ494994	PUNJ14	2002	India: Punjab	Severnini et al. 2002
AJ494995	PUNJ16	2002	India: Punjab	Severini et al. 2002
AJ494996	PUNJ23	2002	India: Punjab	Severnini et al. 2002
AJ494997	PUNJ24	2002	India: Punjab	Severnini et al. 2002
AJ494998	ITA3	2002	Italy Imp.from: Sri Lanka	Severnini et al. 2002
AJ494999	ITA4	2002	Italy Imp.from: Mozambique	Severnini et al. 2002
D85246	T117	1996	Thailand	Putapornitp et al. 1997
D85247	T128	1996	Thailand	Putapornitp et al. 1997
D85248	TD183	1996	Thailand	Putapornitp et al. 1997
D85249	TD201	1996	Thailand	Putapornitp et al. 1997
D85250	TD207A	1996	Thailand	Putapornitp et al. 1997
D85251	TD207B	1996	Thailand	Putapornitp et al. 1997
D85252	TD403	1996	Thailand	Putapornitp et al. 1997
D85253	TD414	1996	Thailand	Putapornitp et al. 1997
D85254	TD424	1996	Thailand	Putapornitp et al. 1997
D85255	TD425	1996	Thailand	Putapornitp et al. 1997
D85256	TD425B	1996	Thailand	Putapornitp et al. 1997
D85257	TD430A	1996	Thailand	Putapornitp et al. 1997
D85258	TD430B	1996	Thailand	Putapornitp et al. 1997
D85259	TD438	1996	Thailand	Putapornitp et al. 1997
D85260	TD439A	1996	Thailand	Putapornitp et al. 1997
D85261	TD439B	1996	Thailand	Putapornitp et al. 1997
D85262	TD452	1996	Thailand	Putapornitp et al. 1997
D85263	TD458A	1996	Thailand	Putapornitp et al. 1997
D85264	TD458B	1996	Thailand	Putapornitp et al. 1997
D85265	V100	1996	Thailand	Putapornitp et al. 1997

Figure 37: MSP-1 gene, deduced amino acid sequence from study samples, references Belém (BELEM) and Salvador I (SALPAT), and sequences available in the Gene Bank. Identical amino acids represented by dots, deletions represented by dashes, unique amino acids in red. Regions are identified as conserved blocks 5 and 6 (CB5 and CB6), recombination segments (RS) I to IV, and conserved region (CR)



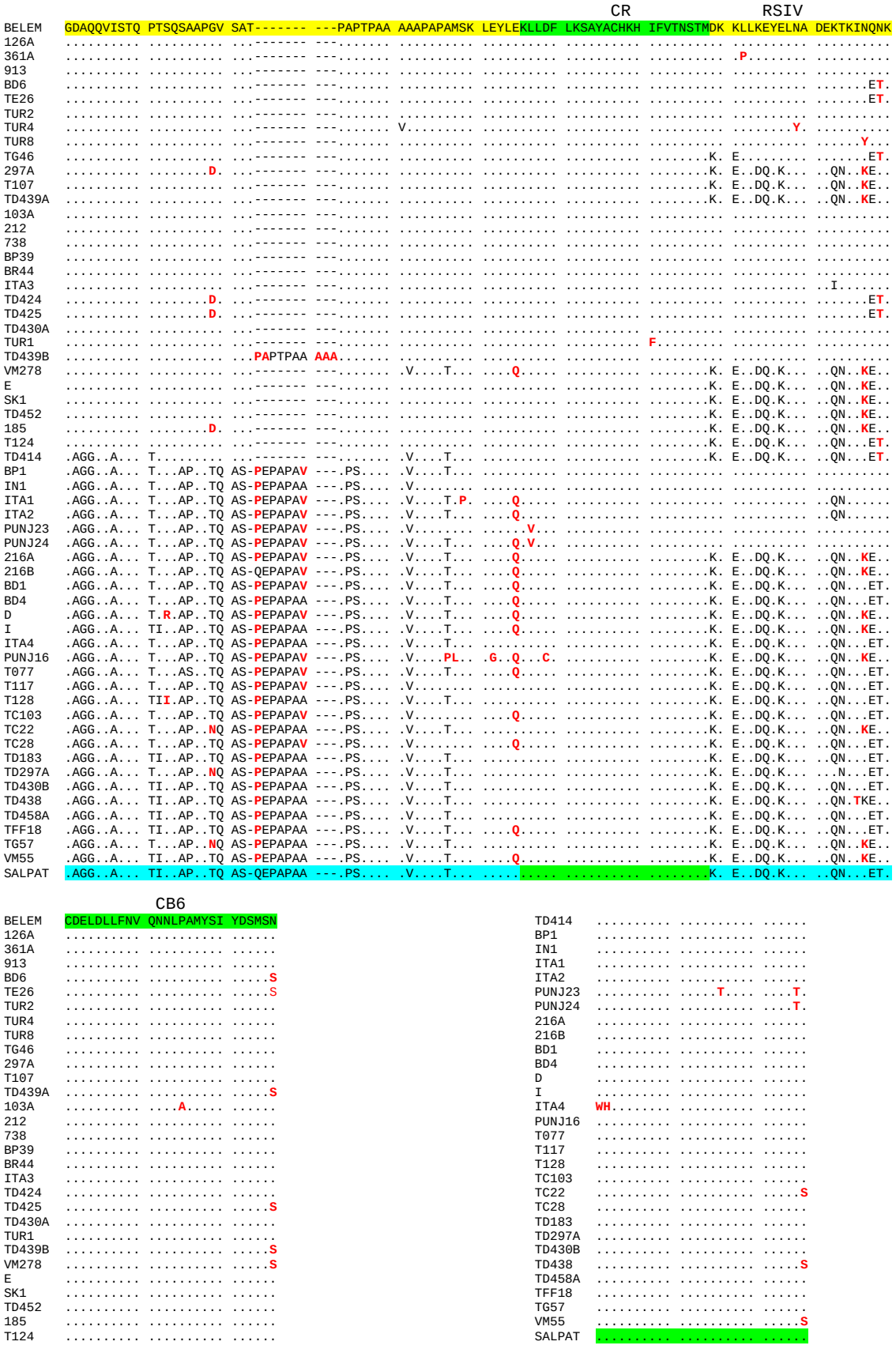
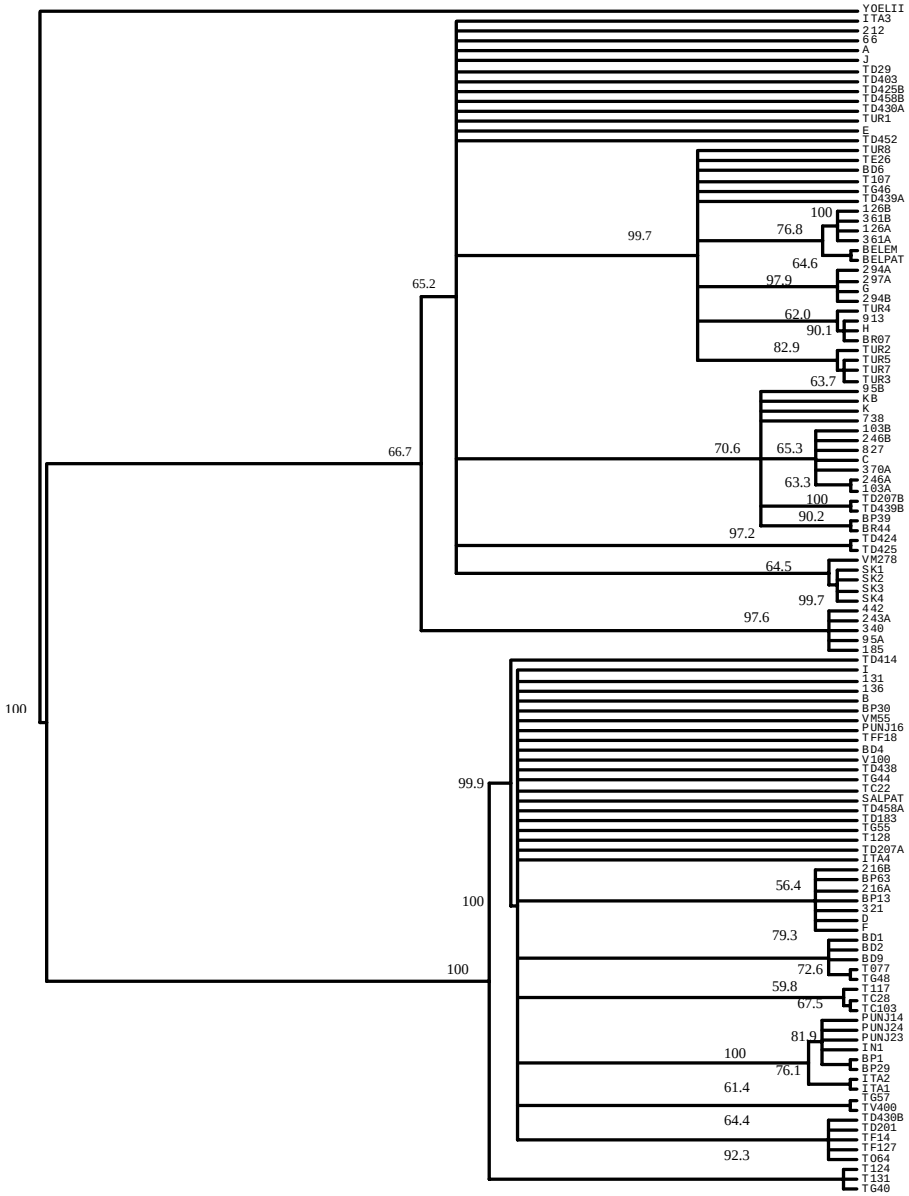

CR
RSIV
CB6

Figure 38: Fifty percent majority rule consensus of trees found by heuristic search showing inferred phylogeny of MSP-1 amino acid sequences of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT) and sequences from Gene Bank. Numbers in nodes are bootstrap support values (cutoff value of 50%).



3.3 Patient Characteristics:

Alecrim (2000) defined two groups of patients in a cohort study at FMT-Am. Patients were either treated in the hospital or followed as outpatients in the malaria clinic. The purpose of Alecrim's study was to describe the clinical and laboratory characteristics of malaria patients and to evaluate anti-malarial drugs. A subset of the patients treated in the malaria clinic presented with secondary infections during treatment and follow-up. As we could not determine if the patients presenting with second waves of parasitemia during follow-up were experiencing relapse or recrudescence infections, this group was named the "relapse group". A sample was randomly selected using the software SPSS from each group of this cohort. Eleven patients were selected from the admitted group, 11 from the no relapse group and 10 from the relapse group.

An identifying code was assigned to each patient, codes for patients in the admitted group consisted of letters, while the codes for those treated as outpatients consisted of numbers. In the case of the relapse group, the letters "A" or "B" were appended to the code numbers, representing the first infection and relapse/recrudescence. From the 32 selected patients, 31 (96.8%) had laboratory data available from primary infections. This information was used to determine if there were any significant differences between the groups and to determine if there was significant association between the genotype of the *P. vivax* strain and the laboratory outcome. Means were compared between groups using Kruskall-Walis and ANOVA. The relationship between variables was determined by the calculation of the correlation coefficients and the cross-

tabulation was tested using X^2-test. All analysis was performed using SPSS version 12.0.1.

3.3.1 Clinical Interview Results:

Sixty-one percent of the total sample was male (19/31) (Table 11). Patient ages ranged from 6 to 60 years old, with a mean of 27.54 (SD: 12.84), indicating predominance of young adults in the sample (Table 12). The distribution by age was similar within each group.

Table 11: Distribution of malaria cases selected for study by sex, relapse status and admission to the hospital.

Sex	Group						Total	
	No relapse		Relapse		Admitted			
	n	%	N	%	n	%	n	%
Female	5	45.4	3	30	4	40	12	38.7
Male	6	54.6	7	70	6	60	19	61.3
Total	11	100	10	100	10	100	31	100

Table 12: Distribution of malaria cases selected for study by age, relapse status and admission to the hospital.

Age Group	Group			Total
	No relapse	Relapse	Admitted	
<10	1	0	2	3
15-20	4	2	1	7
21-25	3	2	2	7
26-35	2	3	2	7
36-50	1	2	3	6
>50	0	2	0	1
Total	11	10	10	31

The patients were administered clinical interviews and asked about their experience with malaria. First-time infection was reported by 61.3% of the patients, 19.4% reported single previous infections, and 19.4% reported two or more previous diagnosis of malaria. In each group, patients experiencing first-time infections predominated (Table 13).

Table 13: Distribution of malaria cases selected for study by history of prior infections, relapse status and admission to the hospital.

Malaria Infection	Group						Total	
	No relapse		Relapse		Admitted			
	n	%	n	%	n	%	N	%
First infection	8	72.7	5	50	6	60	19	61.3
Once	1	9.1	3	30	2	20	6	19.4
Two or more	2	18.2	2	20	2	20	6	19.4
Total	11	100	10	100	10	100	31	100

Patients were also asked about the possible location where they had acquired the infection. The acquisition of the majority of cases was thought to have occurred in the periphery of Manaus, mainly in the east, west and north zones. There was no significant difference in the distribution of patients by group and urban zone. Four cases originated in the following cities: Coari, Nova Olinda, Rio Preto da Eva and Tapauá (Table 14).

Table 14: Distribution of malaria cases selected for study by origin, relapse status and admission to hospital. Origin listed as specific location and zone within the city of Manaus.

Origin of the patients	Group			Zone of Manaus
	No relapse	Relapse	Admitted	
Road AM010 Km30	1	0	0	North
Road BR174 Km10	0	1	0	West
Coari	0	1	0	Other city
Col. Santo Antonio	0	0	1	North
Florestal	1	0	0	North
Jorge Teixeira	0	1	0	West
Mauazinho	1	0	1	East
Monte Das Oliveiras	0	1	1	North
Morada Do Sol	0	1	0	South Central
Nova Olinda	1	0	0	Other City
Novo Israel	0	0	1	North
Parque Das Nações	0	0	1	South Central
Piorini	1	0	0	North
Puraquequara	1	0	0	East
Rio Preto Da Eva	0	1	0	Other City
Santa Etelvina	0	0	1	North
Santa Inês	1	0	0	East
Santa Rita	0	0	1	East
Tapauá	0	0	1	Other City
Tarumã	4	1	1	West
Vivenda Verde	0	1	0	West
Unknown	0	2	1	-
Total	11	10	10	-

3.3.2 Clinical Examination Results:

The attending physician evaluated all patients diagnosed with malaria and collected information on their symptoms. The most common symptoms are presented in Figure 39. There was no statistical difference in the presence or absence of fever, vomit, headache, chills, myalgia, arthralgia, choluria, oliguria, or jaundice within groups. Some of the symptoms were only experienced by patients in the admitted group: one presented with hematuria, another presented with hemolytic anemia and a third one presented with thrombocytopenic purpura. Also, three of the admitted patients required blood transfusions, including the one with hemolytic anemia. Presence of a palpable liver was significantly higher in patients treated in the hospital, X^2-test, $p<0.05$, (Table 15), one patient in the admitted group was splenectomized and there was no data for one patient in the no relapse group.

Figure 39: Distribution of cases selected for study by symptoms, relapse status and hospital admission.

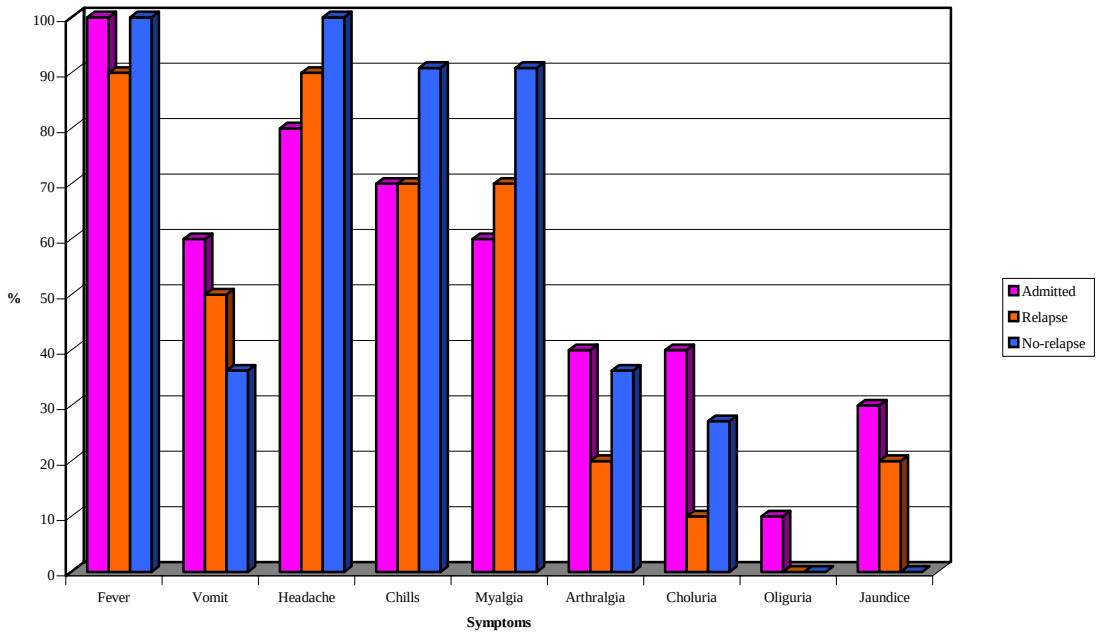


Table 15: Frequency of palpable liver and spleen in patients selected for the study by relapse status and hospital admission.

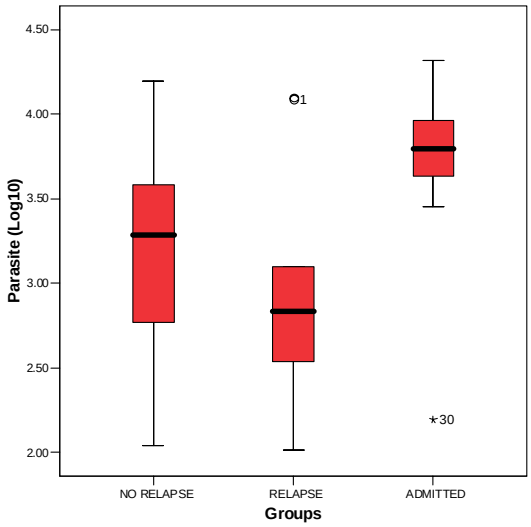
Patient group	Palpable Liver		Palpable Spleen	
	Yes	No	Yes	No
Admitted	8	2	5	4
No relapse	5	6	2	8
Relapse	2	8	0	10
Total	15	16	7	22

$p<0.05$, X^2-test

3.3.3 Laboratory Results:

Trophozoites were counted and expressed as parasites/mm^3 of blood. There was a mean of 4,755.52 parasites/mm^3 (SD: 5,660,09, min: 104, max: 20,862). The admitted patient group had a mean of 7,972.70 (SD: 6,355.13), significantly different from those in the relapse (mean: 2,915.6, SD: 4,989.84) and in the no relapse (mean: 3,503.4, SD: 4,678.91) groups (Figure 40).

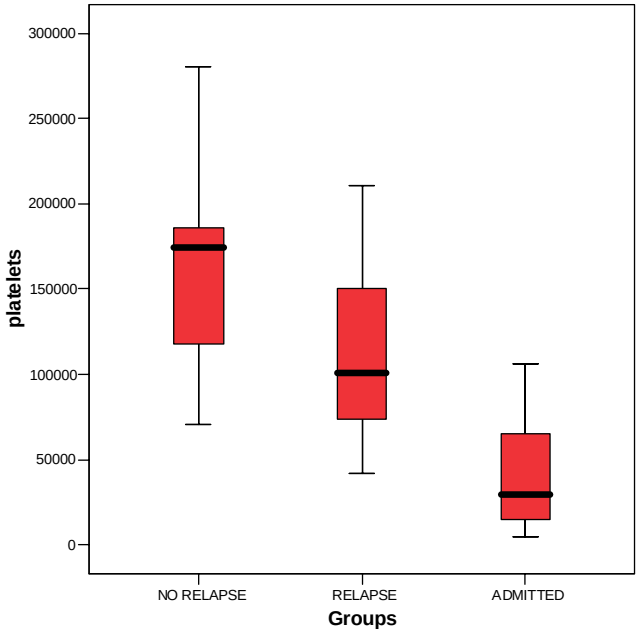
Figure 40: Distribution of the patients included in the study by parasite count (log10) relapse status and hospital admission.



$p<0.05$, ANOVA

Platelet counts ranged from 5,000/mm^3 to 280,000/mm^3, with a mean equal to 102,111/mm^3. Admitted patients had a mean of 42,500 plateles/mm^3, the relapse group had a mean equal to 113,000 platelets/mm^3 and the no relapse group had a mean equal to 158,666.67 platelets/mm^3. Differences between the three groups were statistically significant (Figure 41).

Figure 41: Platelet levels (platelets/mm^3) in the patients included in the study by relapse status and hospital admission.



p<0.05, ANOVA

The mean hematocrit level was 37.11% (SD: 6.53). Patients in the admitted group (mean: 35.61, SD: 8.71) were not significantly different from those in the no relapse (mean: 36.58, SD: 5.64) and relapse (mean: 39.65, SD: 3.89) groups (Table 16). Among the groups, the difference between males (mean: 40.36, SD: 3.23) and females (mean: 32.08, SD: 7.26) was statistically significant at the p=0.05 level (Kruskall-Walis Test).

Table 16: Distribution of cases included in the study by hematocrit levels, relapse status and hospital admission.

Hematocrit %	Group			Total
	Admitted	No relapse	Relapse	
<30	3	1	0	4
31-35	1	2	1	4
35-40	1	3	2	6
>40	5	4	5	14
Total	10	10	8	28

p>0.05, Kruskall-Walis

In the admitted group, the mean hemoglobin level was 11.78 g/dL (SD: 2.69), and was not significantly different from those treated as outpatients. The mean for the relapse and no relapse groups were 13.27 (SD: 1.56) and 12.39 (SD: 1.85) respectively (Table 17). Separating the patients by sex, we found that females had lower hemoglobin levels (mean: 10.83, SD: 2.53) when compared to males (mean: 13.65, SD: 0.91), at a 0.05% significance level (Kruskall-Walis Test). Leukocyte counts (leukocytes/mm^3 of blood) were not different between groups (Table 18) or genders.

Table 17: Distribution of cases included in the study by hemoglobin levels, relapse status and hospital admission.

Hemoglobin (g/dL)	Group			Total
	Admitted	No relapse	Relapse	
<10	3	1	1	5
10-12	2	2	0	4
>12	5	7	7	19
Total	10	10	8	28

p>0.05, Kruskall-Walis

Table 18: Distribution of cases included in the study by leukocyte count, relapse status and hospital admission.

Leukocyte x10^3 (cells/mm^3)	Group			Total
	Admitted	No relapse	Relapse	
3-6	4	6	3	13
6-9	4	3	5	12
9-12	2	1	0	3
Total	10	10	8	28

p>0.05, Kruskall-Walis

There were significant differences in the total and indirect bilirubin levels among treatment groups ($p<0.05$, Kruskall-Walis Test). For the admitted group, the mean level of total bilirubin was 6.12mg/dL (SD: 5.59); in the relapse group, it was 0.42mg/dL (SD: 0.15) and in the no relapse group, it was 0.93mg/dL (SD: 0.88). The indirect bilirubin mean level was 1.55mg/dL (SD: 1.31) in the admitted group, 0.21mg/dL (SD: 0.06) in the relapse group and 0.53mg/dL (SD: 0.64) in the no relapse group.

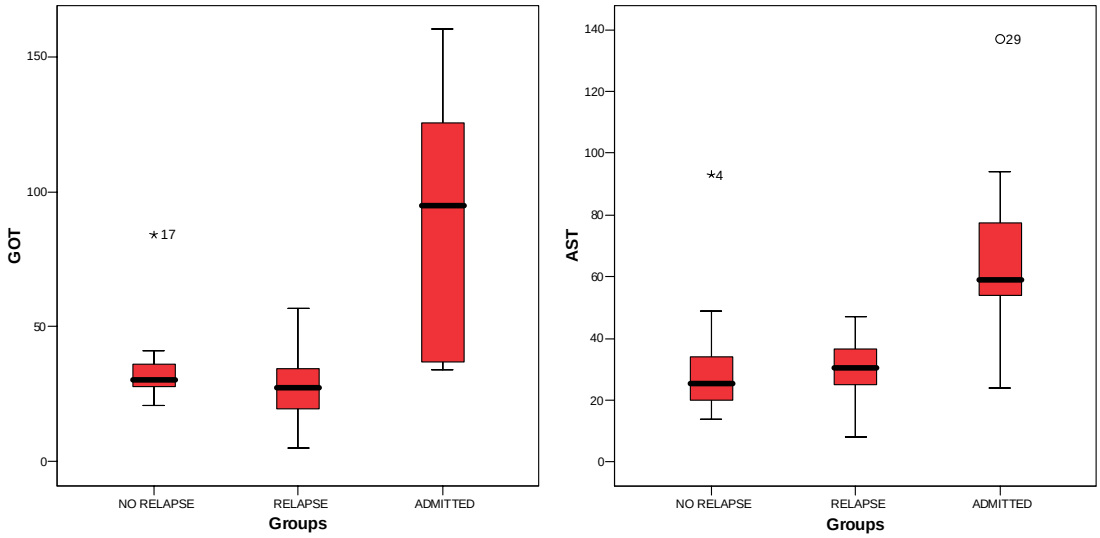
There was no significant difference in glucose levels when the admitted group (mean: 89.1mg/dL, SD: 33.39) was compared to the relapse (mean: 95.8mg/dL, SD: 17.95) and no relapse (mean: 102.3mg/dL, SD: 16.3) groups.

Transaminase levels were higher in the admitted group. Glutamic-oxalacetic transaminase (GOT) means were 87.71U/L (SD: 53) in the admitted groups, 28.12U/L (SD: 15.21) in the relapse group, and 36U/L (SD: 17.69) in the No Relapse group ($p<0.05$) (Figure 41). No significant difference was observed when compared by gender.

The mean aspartate transaminase (AST) level was equal to 69U/L (SD: 36.25) in the admitted group, 29.8U/L (SD: 11.61) in the relapse group, and 32.7U/L (SD: 23.47) in the no relapse group; the difference was significant ($p<0.05$) (Figure 42). No significant difference was observed between males and females.

The mean blood urea nitrogen level was 46.5 mg/dL (SD: 20.24) in the admitted group, 16.55mg/dL (SD: 3.46) relapse group, and 25.86mg/dL (SD: 6.03) in the no relapse group; the difference was statistically significant ($p<0.05$, Kruskall-Walis).

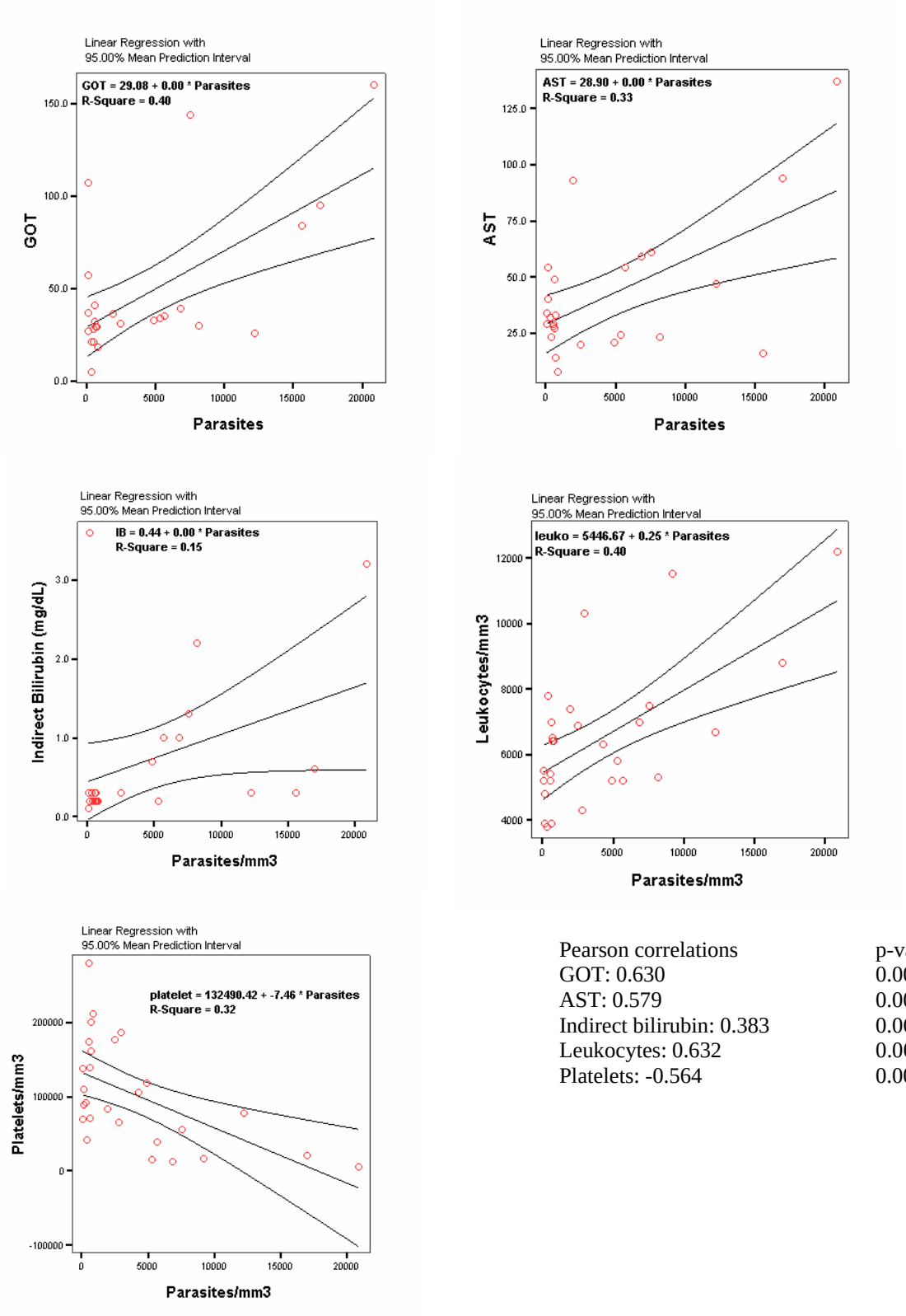
Figure 42: Comparison of cases included in the study by glutamic-oxalacetic transaminase and aspartate transaminase levels, relapse status and hospital admission.



$p<0.05$, Kluskal-Walis

Because most laboratory test abnormalities occurring during malaria infections are related to the effects of the parasite, correlations between parasite level and other variables were calculated. There were significant positive correlations between parasite levels (parasites/mm^3) and glutamic-oxalacetic transaminase levels, aspartate transaminase levels, indirect bilirubin and leukocytes, and a negative correlation between parasite levels and platelet counts (Figure 43).

Figure 43: Correlations between parasite levels and laboratory variables



Pearson correlations	p-value
GOT: 0.630	0.001
AST: 0.579	0.002
Indirect bilirubin: 0.383	0.065
Leukocytes: 0.632	0.000
Platelets: -0.564	0.002

3.3.4 Genotype of *P. vivax* Strains by Patient Characteristics:

3.3.4.1 18S SSUrRNA Type A Genotype and Patient Characteristics:

An A/T single nucleotide polymorphism in the alignment of the SSUrRNA gene was noted, distinguishing the reference strains Salvador I (thymidine) and Belém (adenine). The Salvador I sequence was predominant, at this position, in the admitted group while the no relapse and relapse groups were mainly of the Belém type ($p<0.05$) (Table 19). Comparison of laboratory data revealed that platelet levels were lower in patients infected with parasites with the Salvador I type sequence (mean: 134,000 SD: 60,521.07) when compared to those with the Belém type sequence (mean: 80,187.5 SD: 70,798.51) ($p<0.05$, Kruskall-Walis test).

Table 19: 18S SSUrRNA Type A gene, nucleotide residue 117 sequence distribution by patient group.

	117 Polymorphism		
Patient Group	Belém (A)	Salvador I (T)	Total
Admitted	1	10	11
No Relapse	8	3	11
Relapse	4	6	10
Total	13	19	32

$p<0.05$, X^2-test

Another polymorphism noted in the 18S SSUrRNA Type A gene alignment was the C/T substitution in nucleotide residue 100. There was no significant difference in prevalence of 100:T or 100:C among treatment groups. Analysis of laboratory data revealed that the blood urea nitrogen levels were higher in patients infected with parasites presenting the 100:C polymorphism (mean: 46.0, SD: 13.34), compared to those with 100:T polymorphism (mean: 28.6, SD: 18.41).

Combining information of the two polymorphisms resulted in the formation of four groups (Table 13). There was significant difference in the distribution of patients among these groups but there was no significant difference in their laboratory test means by 18S SSUrRNA Type A genotype. Nine patients, one in the admitted group and eight in the relapse group, had samples from the second time-point infection. Analysis of the nucleotide alignment revealed that two patients had parasites with different 18S SSUrRNA Type A gene sequences, suggesting they presented a new infection and not a relapse/recrudescence of the primary infection. There were no Belém-like strains (117:A) in the South Central zone of Manaus. There was no significant difference in distribution of the strains based on the 18S SSUrRNA Type A gene by urban zone.

3.3.4.2 CSP Genotype and Patient Characteristics:

In all but one isolate, the CSP of *P. vivax* was from the VK210. Sequences from the primary infections included twelve unique sequences (Table 5). Each sequence was representative of a group of strains, each of which was considered to be a sequence group (Annex Figure 1).

Sequence group 1 was identical to the Belém strain (BELPAT), groups 2 and 3 were more closely related to the Belém strain based on the sub-terminal repeat characteristic of Belém, and group four was related to the Salvador I strain, based on the sub terminal repeat characteristic of this strain. Five of the eleven patients treated in the admitted group belonged to the CSP sequence group 1. There was no statistically significant difference in sequence group membership among patient treatment groups (Table 20).

Aspartate transaminase mean levels were significantly higher for patients in the CSP sequence group 2 (Table 21). In the outpatients group, there was a significant difference among CSP sequence groups (Table 22). There was a trend of higher parasite levels and lower platelet levels in sequence group 2 (Figure 44). There was no significant difference in the strains, based on the CSP gene by urban zone

Table 20: Circumsporozoite amino acid sequence distribution by patient group.

Patient Group	CSP sequence group				Total
	Belém	Belém-like 1	Belém-like 2	Salvador-like	
Admitted	5	3	2	1	11
No Relapse	4	0	6	1	11
Relapse	4	0	5	1	10
Total	13	3	13	3	32

$p>0.05$, X^2-test

Table 21: Aspartate transaminase means by CSP sequence group.

CSP sequence group	Mean	Standard Deviation
Belém – sequence group 1	44.4	35.19
Belém like 1– sequence group 2	77.50	23.3
Belém-like 2 – sequence group 3	38.72	21.71
Salvador-like – sequence group 4	12.0	5.65

$p<0.05$, Kruskall-Walis

Figure 44: Parasite and platelet levels by CSP sequence and patient groups.

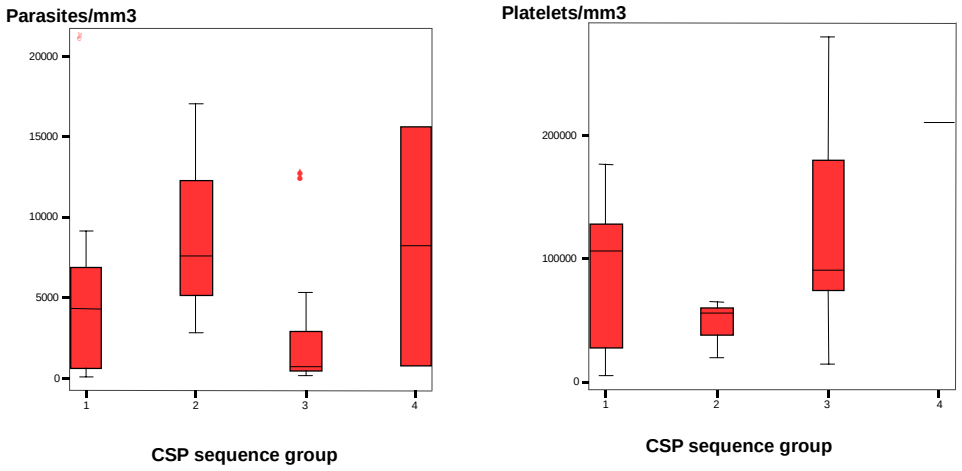


Table 22: Circumsporozoite amino acid sequence group distribution by admitted and outpatient groups.

CSP sequence group	Patient Groups Combined		Total
	Admitted	Outpatients	
Belém – sequence group 1	5	8	13
Belém like 1– sequence group 2	3	0	3
Belém-like 2 – sequence group 3	2	11	13
Salvador-like – sequence group 4	1	2	3
Total	11	21	32

$p<0.05$ X^2-test

3.3.4.3 MSP-1 Genotype and Patient Characteristics:

Based on the MSP-1 gene sequences, the *P. vivax* strains could be divided into three genotypes: Belém-like, Salvador-like and a recombinant, the hybrids. Within the Belém and hybrid groups, there was a subdivision based on the amino acid sequence in the carboxyl terminus of the amino acid sequence. This relationship between strains is represented in the Annex Figure 5. There was no significant difference between MSP-1 group distribution and patient treatment group (Table 23). Most of the Salvador-like strains were from patients in living in the North Zone of Manaus ($p<0.05$, X^2-test).

Table 23: Merozoite Surface Protein – 1 amino acid sequence by patient group.

Patient Group	MSP type					
	Hybrids 1	Hybrids 2	Belém-like 1	Belém-like 2	Salvador-like	Total
Admitted	4	1	1	1	4	11
No Relapse	4	3	0	1	3	11
Relapse	3	2	2	2	1	10
Total	11	6	3	4	8	32

$p>0.05$, X^2-test

A spreadsheet with the data used for analysis is presented on Table 24 and a summary of the descriptive statistics by patient treatment group is presented on Table 25.

Table 24: Spreadsheet from the data used in the analysis:

Status	Paxcode	Origin of infection	Sex	Age	Malaria Infection	Parasite count	Log parasite	GOT	AST
RELAPSE	103 A	IGNORED	Male	47	First infection	12,210	4.09	26	47
RELAPSE	126 A	TARUMA	Male	60	Once	740	2.87	29	33
NO RELAPSE	131	TARUMA	Male	19	First infection	2,950	3.47	-	-
NO RELAPSE	136	TARUMA	Male	24	Once	1,924	3.28	36	93
NO RELAPSE	185	PURAQUEQUARA	Male	15	First infection	8,162	3.91	30	23
NO RELAPSE	212	TARUMA	Male	20	Twice	552	2.74	21	28
RELAPSE	216 A	MONTE DAS OLIVEIRAS	Female	18	Once	144	2.16	57	40
RELAPSE	243 A	JORGE TEIXEIRA	Female	15	First infection	104	2.02	37	29
RELAPSE	246 A	RIO PRETO DA EVA	Male	33	First infection	390	2.59	21	23
RELAPSE	294 A	BR174 KM10	Female	22	First infection	1,254	3.10	-	-
RELAPSE	297 A	IGNORED	Male	43	First infection	630	2.80	32	27
NO RELAPSE	321	FLORESTAL	Female	32	First infection	4,888	3.69	33	21
NO RELAPSE	340	AM010 KM30	Female	22	First infection	110	2.04	27	34
RELAPSE	361 A	VIVENDA VERDE	Male	35	Twice	832	2.92	18	8
RELAPSE	370 A	MORADA DO SOL	Male	22	Once	342	2.53	5	32
NO RELAPSE	442	PIORINI	Female	25	First infection	2,484	3.40	31	20
NO RELAPSE	66	MAUAZINHO	Male	9	First infection	15,600	4.19	84	16
NO RELAPSE	738	SANTA INES	Female	18	First infection	704	2.85	29	14
NO RELAPSE	827	NOVA OLINDA	Male	29	Twice	540	2.73	28	29
NO RELAPSE	913	TARUMA	Female	49	First infection	624	2.80	41	49
RELAPSE	95 A	COARI	Male	34	Twice	12,510	4.10	-	-
ADMITTED	A	PARQUE DAS NACOES	Male	32	Once	5,668	3.75	35	54
ADMITTED	B	NOVO ISRAEL	Male	25	Twice	9,164	3.96	-	-
ADMITTED	C	IGNORED	Male	38	Twice	7,575	3.88	144	61
ADMITTED	D	MONTE DAS OLIVEIRAS	Female	6	First infection	5,336	3.73	34	24
ADMITTED	E	TAPAUA	Male	44	First infection	16,984	4.23	95	94
ADMITTED	F	SANTA ETELVINA	Male	39	Once	6,860	3.84	39	59
ADMITTED	G	SANTA RITA	Female	7	First infection	2,838	3.45	-	-
ADMITTED	H	MAUAZINHO	Female	33	First infection	20,862	4.32	160	137
ADMITTED	I	COL. SANTO ANTONIO	Female	21	First infection	156	2.19	107	54
ADMITTED	J	TARUMA	Male	18	First infection	4,284	3.63	-	-

paxcode	Urea	Creatinine	Glucose	Total Bilirub.	Indi. Bilirub.	Leukocyte	Platelets	Hematocrit	Hemoglobin	Zone of Manaus
103 A	-	-	84.0	0.6	0.3	6,700	78,000	40.1	13.1	-
126 A	-	-	84.0	0.4	0.2	6,500	162,000	39.8	13.4	West
131	-	-	-	-	-	10,300	186,000	36.7	12.8	West
136	19.7	1.4	111.0	-	-	7,400	84,000	41.5	14.1	West
185	-	-	128.0	3.2	2.2	5,300	-	36.6	12.5	East
212	-	-	120.0	0.4	0.2	5,200	174,000	41.2	13.8	West
216 A	-	-	131.0	0.7	0.2	4,800	110,000	41.0	13.8	North
243 A	-	-	106.0	0.2	0.1	5,200	70,000	31.2	9.8	East
246 A	-	-	-	0.4	0.2	7,800	42,000	42.0	13.9	Other City
294 A	-	-	-	-	-	-	-	-	-	West
297 A	-	-	99.0	0.4	0.2	7,000	139,000	44.6	15.3	-
321	35.6	0.9	100.0	1.0	0.7	5,200	118,000	31.9	11.0	North
340	-	-	113.0	0.4	0.3	5,500	138,000	40.8	13.6	North
361 A	19.0	0.6	83.0	0.3	0.2	6,400	211,000	38.1	13.1	West
370 A	14.1	0.5	84.0	0.4	0.3	3,800	92,000	40.4	13.8	South Central
442	24.0	0.9	94.0	0.5	0.3	6,900	177,000	31.5	10.8	North
66	-	-	97.0	0.6	0.3	-	-	-	-	East
738	-	-	71.0	0.5	0.2	6,400	200,000	25.7	8.5	East
827	27.0	0.8	91.0	0.7	0.3	5,400	280,000	44.1	14.6	Other City
913	23.0	0.7	98.0	1.1	0.3	3,900	71,000	35.8	12.2	West
95 A	-	-	-	-	-	-	-	-	-	Other City
A	27.0	0.9	122.0	1.5	1.0	5,200	39,000	40.9	13.8	South Central
B	-	-	-	-	-	11,500	17,000	42.1	13.8	North
C	48.0	1.4	38.0	8.9	1.3	7,500	56,000	34.3	11.5	-
D	58.0	0.9	79.0	0.5	0.2	5,800	15,000	28.9	9.9	North
E	75.0	1.6	130.0	3.7	0.6	8,800	20,000	41.3	13.6	Other City
F	51.0	0.7	87.0	2.1	1.0	7,000	13,000	46.5	13.6	North
G	-	-	-	-	-	4,300	65,000	20.2	6.8	East
H	-	-	-	15.1	3.2	12,200	5,000	42.0	14.7	East
I	20.0	0.5	79.0	11.1	3.6	3,900	89,000	23.9	8.1	North
J	-	-	-	-	-	6,300	106,000	36.0	12.0	West

paxcode	RNA:117 A/T	RNA 100 C/T	RNA 100/117	CSP	MSP	Additional information	RNA+CSP
103a-b	2	2	2	3	1		3
126a-b	1	2	4	3	4		2
131	1	2	4	3	5		2
136	1	2	4	3	5		2
185	1	2	4	1	2		4
212	2	1	3	3	1		5
216a-b	1	2	4	1	5		4
243a-b	2	1	3	1	2		6
246a-b	2	2	2	3	1		3
294a-b	2	1	3	1	3		6
297a-b	2	1	3	1	3		6
321	1	2	3	1	5		6
340	1	2	4	1	2		4
361a-b	1	2	4	4	4		7
370a-b	2	2	2	3	1		3
442	1	2	4	1	2		4
66	2	1	1	4	1		1
738	2	2	4	3	1		2
827	2	2	2	3	1		3
913	2	2	4	3	4		2
95a-b	1	2	4	3	2		2
A	2	1	3	1	1	Hematuria	6
B	1	2	4	1	5		4
C	2	1	3	2	1	Diarrhea, vomit	8
D	2	1	3	3	5	Vomit	5
E	2	2	2	2	2		9
F	2	1	3	1	5		6
G	2	1	3	2	3	Diarrhea, abdominal pain	8
H	2	2	2	1	4	Hemolytic anemia	10
I	2	2	2	3	5		3
J	2	2	2	1	1	Thrombocytopenic purpura	11
K	2	2	2	4	1		12

Table 25: Descriptive statistics of the laboratory data by patient treatment group:

Lab Test	Admitted Group				No Relapse Group				Relapse Group				p-value*
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
Parasites	7972.70	6355.13	156	20862	3503.45	4678.91	110	15600	2915.60	4989.84	104	12510	0.04
Hematocrit	35.61	8.71	20.2	46.5	36.58	5.64	25.7	44.1	39.65	3.89	31.2	44.6	0.689
Hemoglobin	11.78	2.69	6.8	14.7	12.39	1.85	8.5	14.6	13.27	1.56	9.8	15.3	0.455
Urea	46.50	20.24	20.0	75.0	25.86	6.03	19.7	35.6	16.55	3.46	14.1	19.0	0.32
Creatinine	1.00	0.41	0.5	1.6	0.94	0.27	0.7	1.4	0.55	0.07	0.5	0.6	0.161
Total Bilirubin	6.12	5.59	0.5	15.1	0.93	0.88	0.4	3.2	0.42	0.15	0.2	0.7	0.004
Indirect Bilirubin	1.55	1.31	0.2	3.6	0.53	0.64	0.2	2.2	0.21	0.06	0.1	0.3	0.004
GOT	87.71	53.00	34.0	160.0	36.0	17.69	21.0	84.0	28.12	15.21	5.0	57.0	0.007
AST	69.0	36.25	24.0	137.0	32.7	23.47	14.0	93.0	29.87	11.61	8.0	47.0	0.014
Glucose	89.16	33.39	38.0	130.0	102.3	16.30	71.0	128.0	95.85	17.95	83.0	131.0	0.497
Platelets	42500	35094.31	5000	106000	158666.67	64313.68	71000	280000	113000	55092.13	42000	211000	0.001
Leukocytes	7250.00	2832.84	3900	12200	6150.00	1763.36	3900	10300	6025.00	1312.30	3800	7800	0.643

* Kruskall-Walis Test

4. Discussion:

Our primary hypothesis was that the recent epidemic of severe and relapsing malaria in the Amazonas State was due to the emergence of a genetic variant of *P. vivax*. The alternative hypothesis was that the recent epidemic of severe and relapsing malaria in Amazonas State was due to the selection, by poor control measures, for one or more preexisting genetic variants of *P. vivax*.

Malaria in South America represents a major public health problem, especially for countries within the Amazon Region. In Brazil, most of the cases occur in the Amazon Region, particularly in the Amazonas State. *Plasmodium falciparum*, *P. vivax*, and to a lesser extent, *P. malariae*, are the parasites causing the majority of malaria human infections in the region. While *P. vivax* has always accounted for the majority of cases diagnosed and treated in the region, *P. falciparum* was the main cause of severe disease until recently.

Atypical cases of *P. vivax* in Manaus, the capital of the Amazonas State, including patients presenting severe thrombocytopenia and bleeding (Alecrim, 2000; Lacerda et al., 2004), as well as drug resistance (Alecrim et al., 1999) has been described. It was suggested by Alecrim that a subset of the *P. vivax* parasite population could be associated with the occurrence of severe disease (Alecrim et al., 1999, 2000).

To test the hypothesis that severe disease caused by *P. vivax* was associated with a genetic variant, we performed an analysis of the epidemiology of *P. vivax* in Amazonas and conducted a retrospective cohort study. The analyses of *P. vivax* epidemicity clearly documented a substantial increase in the frequency of hospitalizations for *P. vivax*

infections. In the cohort study, we compared patients admitted to the hospital with those treated as outpatients in the FMT-Am. Nucleic acid sequences of three genes from *P. vivax,* the SSUrRNA Type A gene, CSP and MSP-1 genes, were determined. The data were used to describe the characteristics of the strains circulating in Manaus, and to determine if there was an association of genotype with disease severity. We also attempted, unsuccessfully, to determine the nucleotide sequences of the same genes in samples collected 17 years earlier. Our studies yielded substantial information regarding the nature and mechanisms of genetic variation of *P. vivax* in Amazonas, but did not support the hypothesis that a particular genetic variant of *P. vivax* was related to the increased frequency of severe disease in the region. However, our study was not large enough to fully reject the hypothesis. The possible emergence of a *P. vivax* variant with increased pathogenic potential, in the context of an overall increase in the rates of *P. vivax* infections, would have substantial public health significance.

Epidemiology of malaria in the Amazonas State and Manaus:

The Amazonas State of Brazil includes most of the territory of the Amazon Region. The climate and geography of this region as well as the dispersed distribution of the population present challenges for programs aiming to control malaria. The factors that generally contribute to the incidence of severe and fatal malaria include the presence of susceptible populations, aspects of the etiological agents, and the presence and competence of the mosquito vectors. These factors help explain why malaria control programs in the Amazon Region of Brazil have never been completely effective (Silveira & Rezende, 2001; MS-FUNASA, 2002).

We collected data from the Brazilian Ministry of Health and Bulletin reports from the FMT-Am on malaria incidence from 1980 to 2003 for the entire Amazonas State and from 1989 to 2003 for the FMT-Am. We observed that the public health burden of malaria in this region is always present but fluctuates in intensity. In Amazonas State, the rate of malaria per 1,000 inhabitants of the region increased irregularly from 1980 to 2003 with major peaks in 1999 and 2003.

Various strategies have been applied to control malaria in Brazil. The eradication campaign, launched in 1965 was based on DDT spraying and the administration of antimalarial drugs. Although the program was highly effective in eliminating urban malaria and controlling rural malaria, the program was gradually abandoned in the 1980s. Recent programs, which were implemented in response to the WHO's "Roll Back Malaria" effort (Loiola et al., 2002), focused mainly on the early diagnosis and treatment of malaria. With this strategy, the program expects to reduce the number of cases and mortality due to malaria, eliminate malaria transmission from urban areas, ensure the end of transmission in areas where malaria has been interrupted and reduce severe forms of disease (MS-FUNASA, 2002b, Loiola et al., 2002; SVS, 2003). The control measures applied during the period between 1980 and 2003 were not able to control malaria in the State of Amazonas (Figure 8). Malaria rates (cases/1000 population) declined in 2001 and 2002, but in the following years, the rates continually increased.

Among all cases reported in Amazonas, the percent of cases diagnosed in the FMT-Am was relatively constant (Table 1). Most of the cases diagnosed at FMT-Am between 1989 and 2003 were caused by *Plasmodium vivax*, when clinicians observed the increasing numbers of admissions for the treatment of *vivax* malaria.

It is possible that Alecrim's (2000) recruitment of patients to participate in a natural history study between 1997 and 1999 caused an artificial increase in malaria admissions during that time. However, after the conclusion of that study, the number of patients fulfilling the FMT-Am's criteria for admission continued to increase to levels higher than previously observed, especially during the years 2002 and 2003. The criteria for admission included parasite count equal to or higher than +++F or +++V, the finding on thick smear of *P. falciparum* with the presence of schizonts, hypoglycemia, creatinine levels above 1.5 mg/dL, transaminase levels three times the normal level, total bilirubin above 4.0 mg/dL, hematocrit below 21% in adults and below 15% in children, radiologic signs of pulmonary consolidation or diffused infiltrate, and platelet levels below 40,000/mm^3.

To evaluate whether the increased number of admissions due to *P. vivax* malaria represented an epidemic of severe disease, we analyzed the data in various manners. We first defined total malaria and total admissions as the total cases diagnosed in the FMT-Am (*P. falciparum* + *P. vivax* + *P. malariae* + mixed infections) and total cases (*P. falciparum* + *P. vivax*) admitted for treatment, respectively. With the total malaria and total admission as denominators, we calculated the proportion of cases and admissions due to *P. vivax* over the period between 1989 and 2003 (Figure 11). This analysis showed that *P. vivax* was the main cause of malaria, with proportions ranging from 0.70 to 0.93. Admissions due to *vivax* malaria increased significantly from 1997 to 2003, suggesting a change in clinical presentation.

Most of the total malaria diagnosed in the FMT-Am was due to either *Plasmodium vivax* or *P. falciparum*. As shown in Figure 12, we calculated the percent of

admissions for each species and year. We observed a decrease in percent of *P. falciparum* infections resulting in admissions that began approximately in 1991. Control strategies focusing on early diagnosis and treatment have had a greater impact on disease caused by this parasite when compared with *P. vivax* (Mendis et al., 2001, PAHO, 2001). In contrast to the decreased likelihood of admission following diagnosis with *P. falciparum* infection after 1990, there was an increasing likelihood of admission for *P. vivax* malaria after 1996.

FMT-Am is the reference center for diagnosis and treatment of malaria in Amazonas State. We realized that people with severe disease might have been selectively transferred to this hospital for care. Even with the percent of diagnosis in the FMT-Am being almost constant, (Table 1), selective referrals could have produced an artificial increase in the number of admissions. The rate of admissions per 1,000 total population of the Amazonas State is represented in Figure 14. *Plasmodium falciparum* admission rates fluctuated with a decreasing trend during the years of our study period (1989-2003). Fluctuations in *falciparum* malaria admissions could reflect natural variations as well as effects of the control strategies. In contrast, admission rates for *P. vivax* infection in proportion to the total population, increased during the same time-period.

Cohort study and origin of samples:

In the descriptive study carried at the FMT-Am between June 1997 and July 1999, patients were followed over time after diagnosis of infection with *P. vivax* (Alecrim, 2000). Clinical and laboratory parameters were used to determine whether patients would be admitted to the hospital or treated in the ambulatory clinic. Disease manifestations of patients treated in the hospital, included thrombocytopenia, bleeding, thrombocytopenic

purpura, hematuria and DIC, which were not observed in the patients treated in the ambulatory clinic. The main difference reported in the laboratory data was lower platelet counts in the hospitalized patients. Alecrim (2000) suggested that a particular genotype could be the cause for severe symptoms.

In our study, we selected a subset of patients from each of the admitted and outpatient groups to test the hypothesis that genotype was related to the severe manifestation of *P. vivax* infections described in Manaus. To find a difference of approximately 50% in the prevalence of a particular genotype among strains affecting the groups, with a power of 80%, 30 samples were required (ten admitted cases and twenty outpatient controls). We randomly selected 32 samples from the list of patients in the Alecrim cohort. One extra sample for each group was selected in the event that problems with DNA amplification by PCR were encountered.

Alecrim (2000) defined severe *vivax* malaria as classic signs and symptoms of malaria associated with one or more of the following complications: jaundice with total bilirubin equal or higher than 5mg/dL, anemia with hemoglobin below 6mg/dL and hematocrit equal or below 20%, convulsive crisis, hypoglycemia, platelets below 49,000/mm^3, bleeding, coma, shock, hemoglobinuric fever, spleen rupture, and pulmonary alterations. The admission criteria included patients with severe or moderate disease and excluded patients with co-morbid chronic diseases, such as diabetes.

In the subset of patients selected for this study, the admitted group presented with higher mean parasite counts, lower platelet counts, and higher levels of liver enzymes, total and indirect bilirubin and urea than those treated in the ambulatory clinic. Symptoms of severe disease, including hematuria, hemolytic anemia, and thrombocytopenia were

only noted in the admitted group. Presence of palpable liver was more frequent in patients treated in the admitted group.

Laboratory and clinic data were analyzed for ten of the eleven patients in the admitted group. Based on the criteria adopted by Alecrim (2000), nine out of ten patients in the admitted group presented with at least one of the criteria for severity, one presented with a hemoglobin level of 6.8mg/dL, and one was pregnant. In the non-admitted group, one patient was presented with a platelet count bellow 49,000/mm^3 and none were pregnant.

Atypical and protean manifestations of *P. vivax* infection have also been reported in India where the parasite is also the main cause of malaria. Mohapatra and collaborators (2002) described jaundice, cerebral involvement, severe anemia, thrombocytopenia and pancytopenia in a cohort of 110 patients enrolled to study atypical presentations and chloroquine sensitivity. Another study from India (Kochar et al., 2005) described eleven cases of severe *P. vivax* infection causing both sequestration-related and nonsequestration-related complications. Diagnosis was confirmed by PCR targeting the 18S SSUrRNA gene. Complications included cerebral malaria, renal failure, circulatory collapse, severe anemia, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome, and jaundice. Parasitemia ranged from 8,000 to 90,400/mm^3, two patients were pregnant, one was in the puerperal period, and two patients died. In Korea, (Oh et al., 2001) the only complication was a splenic rupture in one patient.

Patient characteristics:

Our study sample was mainly composed of young males with no prior immunity to malaria, as they reported that they were experiencing infection for the first time. Most of the infections were acquired in the periphery of Manaus, zones North, East and West, an area of poor living conditions, and in many cases close to forested areas.

Malaria disease results from infection with the asexual blood form of the parasite (White, 1998). The laboratory profile of patients with malaria is used to estimate the severity of disease. In *P. vivax*, infection is limited by the fact that the parasite only invades reticulocytes, and not mature erythrocytes. Anemia, neutropenia, thrombocytopenia, lymphopenia and monocytosis may occur during *P. vivax* infection (Wickramashinghe & Abdalla, 2000).

Various factors contribute to the pathogenesis of anemia during malaria infection, destruction of red cells during schizogony, destruction of nonparasitized cells, and reduced threshold for splenic clearance of abnormal erythrocytes. The role of antibody in Coomb's-positive hemolysis is unresolved (White, 1998). In our study samples, there was no difference between hematocrit (Table 16) and hemoglobin (Table 17) levels among patient treatment groups. One patient in the admitted patient group and one in the no relapse group presented with hemoglobin equal to 8.1 and 8.5g/dL, respectively. In severe cases from India, 63.6% (7 of 11) presented with hemoglobin levels equal to or less than 8g/dL (Kochar et al., 2005).

Thrombocytopenia occurs in both *P. falciparum* and *P. vivax* infections. It is caused by consumption of platelets by coagulation processes, increased platelet turnover and splenic clearance, there is also evidence for platelet activation (White, 1998).

Profound thrombocytopenia is usually associated with disseminated intravascular coagulation (DIC) related to *P. falciparum* infection (White, 1998) but it has been reported during infections caused by *P. vivax* (Anstey, Currie, Dyer, 1992; Horstmann et al., 1981; Kakar et al., 1999; Kelton et al., 1983; Looareesuwan et al., 1992; Makkar et al., 2002; Yamaguchi et al., 1997; Lacerda et al., 2004). Direct interaction between parasites and platelets has been suggested because *Plasmodium vivax* was observed inside platelets (Fajardo & Tallent, 1974; Horstmann et al., 1981). Platelet-associated IgG has been linked to thrombocytopenia in some studies (Yamaguchi et al., 1997; Kelton et al., 1983), but not in others (Looareesuwan et al., 1992). In our study, hospital patients presented significantly lower platelet counts (Figure 41) when compared to outpatients. Sixty percent (6 of 10) of the patients in the admitted group presented with severe thrombocytopenia (<50,000/mm^3). In Korea (Oh et al., 2001) severe thrombocytopenia (<60,000/mm^3) occurred in 29.7% (30/101) of patients. In India (Kochar et al., 2005), one out of eleven patients presenting with severe disease had platelet count equal to 50,000/mm^3.

During *P. falciparum* infection, total and indirect (unconjugated) bilirubin are increased consistent with hemolysis. Serum aminotransferases are moderately elevated (Warrel, 1996). Patients in our admitted group presented higher total and indirect bilirubin levels and higher AST and GOT levels when compared to those treated as outpatients. In India (Kochar et al., 2005), 36% (4/11) and 45% (5/11) of the patients evaluated presented with elevated aminotransferases and bilirubin levels, respectively.

In our test samples, there was a negative correlation between platelets and parasite counts (Figure 43). This same type of correlation was observed by Horstmann and

collaborators (1981). These authors also found reduced life span of platelets in a study of three patients. Positive correlation was found between platelet counts and total and indirect bilirubin, AST and GOT levels (Horstmann et al., 1981).

Ten of the eleven patients in the admitted group presented with 117:T polymorphism in the 18 S SSUrRNA Type A nucleotide sequence. While most of the outpatients presented with the 117:A polymorphism, this difference was statistically significant (Table 19). Platelet levels were significantly lower in the patients presenting with 117:T. The second most common polymorphism was at residue 100 but there was no frequency predominance by patient treatment groups. Phylogeny of the 18S SSUrRNA gene fragment was mainly a reflection of the polymorphisms at residues 100 and 117 (Figure 19). Patients were allocated into four main groups. There was no significant bootstrap support value for these groups and the tree may not be reflective of the phylogeny of the organism.

All patients in the study were infected with the VK210 CSP variant of *P. vivax*. Amino acid sequence was used to determine diversity within this variant. Fifteen unique sequence groups were defined for the strains isolated from our samples (Figure 27). Accumulation of point mutations in the repeat region was the main cause of sequence diversity. Phylogenetic analysis revealed the relationship between the strains from Manaus and references Salvador and Belém. Five patients in the admitted group presented with amino acid sequences identical to the reference Belém, five presented with amino acid sequences similar but not identical to the Belém strain, based on repeat block 20. One patient in the admitted group presented with an amino acid sequence more closely related to the Salvador I strain. Patient distribution by treatment group and CSP

sequence group was statistically significant (Table 22). There was a trend for low platelet counts (Figure 44) and a high AST levels (Table 21) associated with one of the Belém-related amino acid sequences.

Phylogenetic Methods:

We analyzed both nucleotide and deduced amino acid sequences of *P. vivax* genes. Two of the three genes studied were protein-coding genes. Variation in sequence diversity at the nucleotide level could affect the phylogeny and we considered amino acid sequences in the analysis for comparison of the topology. Sequences were first aligned in the computer and then edited manually to minimize gaps and to avoid the separation of codons.

We used distance and character-based approaches to infer the phylogeny of *P. vivax* genes. The distance-based approach computes evolutionary distances for all pairs of taxa and constructs the phylogenetic tree by considering the relationship among these distance values. In the character-based approach, each character is analyzed separated and usually independently. The topology that requires the smallest number of substitutions that explain the evolutionary process is chosen to be the best tree (Nei & Kumar, 2000; Salemi & Vandamme, 2003; Felsenstein, 2004). Bootstrap was used as the statistical estimate of the reliability of the groups formed.

There are several methods to construct phylogenetic trees within each criterion of analysis (Nei & Kumar, 2000; Salemi & Vandamme, 2003; Felsenstein, 2004). Based on the literature and on the characteristics of our data, we selected the neighbor-joining and heuristic search methods to construct the trees. In the neighbor-joining tree search method, we applied several algorithms to calculate the distance among taxa (PAUP

scripts annexed). Each algorithm was based on a different evolutionary model and each tree was produced individually. We combined all distance-based trees in a consensus and looked for the tree that best fit our sequence data.

Heuristic search under parsimony with stepwise addition, sequences were added at random and tree bisection and reconnection was the branch-swapping algorithm. This process was the most suitable to our number of taxa and alignment size. Using this method, several trees were formed and combined in a consensus. We used the sequence alignments to determine if the consensus tree reflected the characteristics of our data.

Historical samples for study of the phylogeny of P. vivax:

The original proposal in this study included, as one specific aim, the comparison of the strains isolated from the recent epidemic of malaria caused by *P. vivax* with samples from 1970s stored as historical blood smears. Contamination of the slides with fungal and bacterial genomic material made it impossible to complete this aim. As a result, we did not have historical sequences from patients infected in Amazonas with which to compare recent sequences. Our approach to overcome this problem was to compare study sequences to older sequences isolated from Brazil (Belém) and Central America (Salvador I). These strains have been used as the reference strains in several studies (Arnot et al., 1988; Arnot et al., 1990; Cui et al., 2003b; Kolakovich et al., 1996; Lim et al., 2000; Lim et al., 2001; Leclerc et al., 2004; Maestre et al., 2004; Mancilla et al., 1994; Porto et al., 1992; Premawansa et al., 1993; Putaporntip et al., 1997; Putaporntip et al., 2002; Severini et al., 2002; Severini et al., 2004). Dr. Barnwell provided both reference strains, Belém and Salvador I, kept in the laboratories of the Center for Diseases Control in Atlanta. The Salvador I strain was isolated from a human

patient in 1969 in El Salvador and the Belém strain was isolated in a patient from Brazil in 1980 (Barnwell, personal communication). The cohort study from which the strains of *P. vivax* that were included in our phylogenetic analyses were obtained was carried out in the Amazonas State of Brazil between June 1997 and July 1999. We also compared sequences of *P. vivax* available in the Gene Bank to our sequences. Since historical samples from Amazonas were not available, these reference sequences were the best option for use in our study.

Malaria Species and Subtypes in Cohort Patients:

In the Amazonas State, Brazil, *P. vivax*, *P. falciparum* and *P. malariae* are endemic. Our first step was to analyze all samples by PCR to confirm the unique presence of *P. vivax* in the samples using a PCR targeting the 18S SSUrRNA Type A gene. There were no mixed infections with the other malaria species. The 18SS SUrRNA Type A gene was also studied for phylogenetic analysis.

We identified two variants of *P. vivax* based on the CSP gene sequences. VK210 was present in all patient strains, and *P. vivax*-like was identified as a mixed infection with a VK210 strain in one case. Amino acid sequence analysis revealed 15 sequences of *P. vivax* VK210 among the samples, most of them more closely related to the Belém reference strain than the Salvador I strain. Point mutations, insertions and deletions were the main source of genetic diversity observed in this gene.

The MSP-1 gene segment examined in our samples revealed the presence of three basic amino acid sequence groups, one related to the Belém strain, a second related to the Salvador strain, and a third form was a recombination between progenitors related to the two reference strains. Four recombination sites were identified in the MSP-1 sequence

between interspecies conserved blocks 5 and 6. Sequence variation was primarily caused by recombination and, to a lesser extent, by the alteration of amino acids by point mutations within each region.

The sample population included patients with second time-point infections (relapse group). Genetic data from the second infection were not analyzed to determine relationship to disease severity but were used for phylogentic analyses and for the examination of within-patient evolution.

18S SSUrRNA gene Type A:

We examined the segment between variable regions 7 and 8 from the 18S SSUrRNA Type A gene in samples from Amazonas State, Brazil. These strains were compared to each other, to the reference strains Salvador I and Belém and to additional strains obtained from the Gene Bank. We found limited variation in the DNA sequences analyzed. Several polymorphisms were found to be present in only one or a few samples. Two polymorphisms were noted to occur at higher frequency. Ten strains from Manaus were found to have a C→T transition at residue 100 of the segment. This same mutation was found in the SAL I (U03079) strain from the Gene Bank. Among the strains with 100:C, five were treated in the hospital and five were treated as outpatients. Thymidine at residue 100 was found in six of the eleven patients treated in the hospital and 16 of the 21 patients treated in the clinic.

A second frequent polymorphism was noted at position 117. We observed that 10 of 11 patients in the admitted group compared to 13/21 in the outpatient group were 117:T. This frequency difference was statistically significant ($p<0.05$). The Salvador I strain was T at this position and the Belém strain was A.

The construction of phylogenetic trees using the alignment of 18S SSUrRNA Type A gene yielded four main groups (Figure 19), mainly based on the nucleotide 100/117 polymorphisms. The parsimony approach used to construct the tree takes into account variable sites that are parsimony informative, e.g., those sites with at least two different kinds of nucleotides, each represented at least twice (Nei & Kumar, 2000). Using the bootstrap method to estimate the variability of an estimate, the nucleotide alignment was resampled with replacement, to produce a fictional sample of the same size (Felsenstein, 2004). In the case of the 18S SSUrRNA Type A gene sequence data, there were a limited number of variable sites limiting the significance of the bootstrap support values.

Additional mutations were found at residues 118 (strain 66), 119 (strains A and J), and 124 (Strain C). Thus, mutations were found at four of the eight residues between residues 117 and 124, suggesting that this region may have been relatively hypervariable. Other gene regions with relatively high numbers of mutations were noted in residues 58 to 75 with three variant residues, and between 301 and 325, also with three variant residues.

Comparison of the strains from Manaus with other strains from various geographic regions was part of the methodology to study the phylogeny of *P. vivax*. Four additional sequences representing strains from three geographic regions were retrieved from the Gene Bank. The strains from Colombia and Thailand were identical to the one from Belém, Brazil (BELPAT). The two additional strains from El Salvador were different from our reference strain (SALPAT) in some residues but had the characteristic 117:T that differentiates the El Salvador from the Belém (117: A) strain.

Intra-species DNA sequences of SSUrRNA gene have been previously described for *P. vivax* (Alecrim et al., 1999, Li et al., 2001), *P. ovale* (Kawamoto et al., 1996), and *P. malariae* (Liu et al., 1998). In the study by Alecrim et al. (1999), which proposed the emergence of a pathogenic genetic variant, one strain isolated from a patient with relapsing disease from Manaus was found to have four polymorphisms in the 18S SSUrRNA Type A gene, when compared to strains from El Salvador and Thailand. None of the samples in our study showed similar mutations.

Circumsporozoite Surface Protein Gene:

The structure of the CSP gene of *P. vivax* is that of a central variable region, coding for repetitive amino acid blocks flanked by conserved regions I and II. There are three variants of *P. vivax* CSP based on the central region, VK210, VK247 and *vivax*-like, described in the literature (Arnot et al., 1985; Rosemberg et al., 1989; Qari et al., 1993a). Variation in the nucleotide and amino acid sequences within the three CSP variants have been observed to occur in various regions of the world (Arnot et al., 1990; Kho et al., 1999; Kim et al., 2002; Lim et al., 2001; Mann et al., 1994; Qari et al., 1991, 1992; Rongnoparut et al., 1995), including some areas in Brazil (Qari et al., 1991, 1992). This present study is the first description of DNA and deduced amino acid sequences of strains isolated from the Amazonas State of Brazil.

The protocol used to amplify the gene encoding the CSP gene was designed so that the primers would be useful for all three strains. The suitability of these primers was confirmed using plasmid DNA representative of the three variants. An RFLP technique was tested as a method to determine CSP genotypes, based on differences in restriction enzyme recognition sequences between VK210, VK247, and *vivax*-like sequences.

Application of the protocol to our study samples revealed mixed infections, identified as double bands on agarose gels, possible variations in the repeat numbers, and variations within the VK210 group, identified as various digestion patterns of the PCR products (Figure 23).

All of the patients in this study were infected with the VK210 strain of *P. vivax*. Nucleic acid sequencing of the PCR products that yielded two bands on gel electrophoresis revealed that one case was due to a mixed infection of VK210 strains, and another was due to a mixed infection of VK210 and *P. vivax*-like strains. The variant VK247 was not detected in the test samples. All three forms of *P. vivax* CSP have been detected in samples from Brazil, outside of the Amazonas State, using ELISA (Curado et al., 1997; Qari, 1193a), GFM-PCR-ELISA (Machado et al., 2000, 2003), and by PCR and sequencing of the CSP gene (Qari et al., 1991, 1992, 1993b). In these other reports, VK247 and *vivax*-like strains were found as mixed infections with VK210 strains. In contrast, our results revealed the VK210 strain as the sole source of infection in all but one case.

Alecrim (2000), using PCR followed by hybridization, detected both VK210 and VK247 in test samples from Manaus. It was also noted that some of the strains did not hybridize with DNA of either VK210 or VK247. The author did not use probes for *P. vivax*-like, but it is possible that hybridization did not occur due to an increased nucleotide divergence between the VK210 strains. Variability between strains has been noted as the cause of reduced sensitivity in immune-enzymatic assays (Rongnoparut et al., 1995). In a study by Alecrim (2000), VK247 was only detected as mixed infection with VK210, and the author concluded that VK210 was solely responsible for the clinical

manifestations observed in the patients from whom samples originated. In samples from Belém, Pará State, Brazil, VK210 was also identified as the type most strongly correlated with the classic symptoms of malaria (Machado et al., 2003), but none of the other variants were presented as the sole source of infection.

An RFLP analysis was used to determine the level of variation within CSP subtypes in strains from Korea. Two VK210 genotypes were identified (Kho et al., 1999). In test samples from Manaus, size variation of the PCR products and different patterns of digestion suggested the presence of various VK210 subgroups. This interpretation was confirmed by DNA sequencing.

The deduced amino acid sequences of each of the test samples were analyzed in relation to the reference strains Salvador I and Belém, both VK210, and other sequences available in Gene Bank. The variable region of the references Salvador I and Belém comprised 21 repeat blocks composed of nine amino acids each (Figure 24). Common repeat blocks GDRADGQPA and GDRAAGQPA occurred between repeat blocks 1 to 19, amino acid block 20 was GNGAGGQAA for the Belém strain and GDRAAGQAA for the Salvador I strain, and the 21st repeat block, GNGAGG(G)QGQ was common for both strains.

Fifteen unique VK210 sequences were identified in the test samples from Manaus (Table 6, Figure 27). The number of repeat blocks varied from 19 to 21, the amino acid composition included the repeat blocks present in the reference strains and a variant block GNRADGQPA in repeat block six of the sequence groups 11 and 12 (Figure 27). Two amino acid sequence groups contained alanine insertions amino to the first repeat block. In two VK210 clones isolated from test sample C, the variant repeat blocks

GDRTDGQPA and GDRAARQPA were observed in blocks 11 and 15, respectively (Figure 32).

Sequence features that have been reported by others (Rosemberg et al., 1989; Rongnoparut, 1995; Mann et al., 1994; Leclerc et al., 2004) were also noted in our studies. An insertion of the codon GCA, alanine, was noted immediately prior to the first repeat block, in four of the forty-one sequences from Manaus, including two from patients admitted to the hospital for treatment, test samples C and E, and two from individuals treated as outpatients, test samples 126A and 297B. This polymorphism has also been observed in sequences from Thailand (Rosemberg et al., 1989; Rongnoparut, 1995), The Philippines, The Solomon Islands and China (Mann et al., 1994), and Azerbaijan (Leclerc et al., 2004).

Also noted in our strains was a bias towards the use of the nucleotide adenine in the third position, as noted in the codons of both alanine and glycine described by Arnot and collaborators (1988). Contrary to other published sequences of CSP *P. vivax* genes (Qari et al., 1991, 1992; Rongnoparut et al., 1995; Rosemberg e al., 1989; Qari et al., 1993b; Mann et al., 1994; Arnot et al., 1985), we found CCT coding for proline in the strain identified as A (7^{th} amino acid of blocks 2 and 8).

Frequent synonymous substitutions in codons 1, 2 and 7 were also observed in strains from various geographic regions (Qari et al., 1992; Mann et al., 1994; Rongnoparut et al., 1995; Lim et al., 2001; Kim et al., 2002; Leclerc et al., 2004) suggesting that this is a characteristic of the CSP gene from *P. vivax*. Synonymous substitutions in codons 6, 8 and 9 were less frequent.

The variant repeat GNRADGQPA, observed in block 6 of the VK210 sequence groups 11 and 12, was also present in clones from Brazil described by Qari et al (1992). Some of their sequences were identical to those found in this study: Clone B5/1 had the same amino acid sequence as our test samples with VK210 sequence 12, clone B26/1 was identical to sequence 11, clone B30/20 was identical to sequence 1 and clone B13/1 was identical to sequence 4.

Other variant repeat blocks, insertions and/or deletions of one or various amino acids in the central variable region of the CS protein are described in the literature (Arnot et al., 1990; Mann et al., 1994; Rongnoparut et al., 1995; Lim et al., 2001; Kim et al., 2002; Leclerc et al., 2004). Mann et al. (1994, 1995) raised questions about the integrity of variable repeats. They observed that the insertion of *in vitro* artifacts during the process of PCR amplification and cloning explained the presence of multiple non-synonymous substitutions generating the variation of CSP strains. In our study, we used polymerases with high specificity during the PCR technique, and performed direct sequence of our amplified products.

Amino acid substitution was frequent in the fifth position of repeat blocks 1 to 19 (Figure 27); synonymous substitutions were never present in this codon. Substitutions in the second (D/N) and eighth (P/A) codons were less frequent and were comparable to the work of Qari et al. (1992). The frequent alternation between alanine (A) and aspartic acid (D), observed in codon 5 of the repeat blocks in our strains, is a common event in the CSP of *P. vivax*, with a strong bias towards use of the codons GCT and GAT (Qari et al., 1992; Leclerc et al., 2004; Mann et al., 1994; Rongnoparut et al., 1995). The biochemical characteristics of alanine and aspartic acid are such that this substitution could cause

major impact on protein function. Alanine is a non-polar, hydrophobic amino acid, while aspartic acid is negatively charged, acidic, and hydrophilic. Charged amino acids in the conserved region II of the CSP are important to the binding of sulfated chains in hepatocytes during the process of cell invasion (Nardin & Zavala, 1998). A function associated with the amino acid at the fifth position of the *P. vivax* CSP repeat block has not been defined. It is known that antibodies against the CSP target the repeat region (Bilsborough et al., 1997; Nardin & Zavala, 1998) as peptides from this region induce lymphocyte proliferation in both mice and humans (Romero et al., 1987; Herrera et al., 1992), and that amino acid composition of the peptides seems to be essential for recognition by mAb (Romero et al., 1987). HLA-A2-restricted $CD8^{+}$ epitopes were identified in the conserved regions of the CSP; these epitopes were recognized by lymphocytes from Colombian individuals (Arévalo-Herrera et al., 2002). Peptides from different regions of the CSP have been used in studies evaluating the CD8+ response during infection with *P. falciparum* (Malik et al., 1991; Sedegah et al., 1992; Wizel et al., 1995). We found no published studies using the repeat blocks of *P. vivax* as epitopes for CD8+ T-cells. Based on the literature and our findings, we can suggest that the frequent substitution of alanine with aspartic acid is probably important for parasite immune evasion mechanisms, and that response to this same peptide may vary among different populations.

Heuristic search under the parsimony criterion, using the amino acid sequence alignment (Figure 30), combined the 15 VK210 amino acid sequence groups into three main clusters. One group with significant bootstrap support (80.7%) was composed of the strains with amino acid sequence group 1. The other main cluster was composed of

sequences related to the Salvador I strain, consisting of amino acid sequence groups 4, 5, 6 and 15. Subdivision of this group yielded two statistically significant subgroups, one composed of sequence groups 4 (66), 5 (361A) and 15 (361B, 370B) and another composed of sequence group 6 (test sample K). The third main group defined by heuristic search was composed of amino acid sequence groups 2, 3, 7, 8, 9, 10, 11, 12, 13, and 14. The inclusion of test sample 246A in this group was not supported by bootstrap analysis; all other strains were allocated to this group 96.4% of the time. Test samples with amino acid sequences 2 (G), 3 (C, E, 297B) and 14 (95B) formed a significant subgroup. All these amino acid sequences were related to the Belém strain, as they contained the characteristic amino acid of the second to the last repeat block of the Belém strain.

Distance analysis of the amino acid alignment (Annex Figure 1) best represented the subdivision of the VK210 subtypes. The similarities between VK210 subtypes were represented; allocation of the groups was based on the number and type of repeat blocks, and relationship to the reference strains Salvador I and Belém. Heuristic search and neighbor-joining amino acid sequence trees were similar in distributing the strains among groups and subgroups. The high frequency of synonymous substitutions within each amino acid sequence type was represented in the phylogenetic trees built using nucleotide sequences (Annex Figures 2 and 3).

Sequences corresponding to the same segment of the CSP gene were retrieved from Gene Bank. Most of the sequences were from Asia and presented characteristics not observed in the sequences from Manaus, such as insertion of amino acid segments. Limited availability of sequence data from other publications limited this analysis.

Merozoite Surface Protein-1:

MSP-1 sequences from different malaria species demonstrated the presence of conserved, semi-conserved and variable blocks (Del Portillo et al., 1991; Cooper, 1993; Gibson et al., 1992; Putaporntip et al., 2002). The variable region between interspecies conserved blocks 5 and 6 was selected to study the molecular epidemiology of *P. vivax* in the Amazon Region of Brazil. Our studies of this gene region revealed no evidence of mixed infection in any assay result.

Forty sequences were analyzed from 11 patients treated in the hospital, and 21 patients treated in the ambulatory clinic, 10 of whom presented with second time-point infections. Our results indicated that the main source of variation in this gene segment was recombination among progenitor strains closely related to the Salvador I and Belém strains, as observed by other authors (Premawansa et al., 1993; Putaporntip et al., 2002). This interpretation was supported by both inspection of sequence alignments and phylogenetic analysis. Similar recombination was also observed in other sequences retrieved from the Gene Bank (Putaporntip et al., 1997 and 2002), supporting the hypothesis that recombination events not only generate diversity but can also occur at various sites along the MSP-1 molecule (Putaporntip et al., 2002).

Our results differed from those of Kolakovich et al. (1996) in which Salvador I-like MSP-1 proteins were present in more than 90% of strains from Papua New Guinea. Our phylogenetic analysis, which included sequences from various regions of the world, supported the interpretation that global diversity of the gene derives from recombination between Salvador I- and Belém-like progenitors. In the comparison of our strains with strains from the Gene Bank we observed a variety of strains clustering with ours. These

strains were from regions as distant as Korea and Thailand. Sequences isolated from Brazil were available in Gene Bank, some were related to our strains while others were identical (Putaporntip et al., 2002).

The second most significant form of variation was in the polyglutamine region of strains with Belém-like sequences in the central part of this gene segment. The number of glutamine residues in the central region of the sequences Belém-like ranged from 15 to 30. In sequences with recombinant sequences, the number of glutamines was between 18 and 21. Variation in the number of glutamine residues was the main variation reported in various other studies (Premawansa et al., 1993; Putaporntip et al., 1997; Lim et al., 2000; Putaporntip et al., 2002; Severini et al., 2002; Maestre et al., 2004; Severini et al., 2004). Results by Oliveira et al. (1999) demonstrated that the polyglutamine residue was highly immunogenic.

Apart from recombination and variation in the length of polyglutamine tracks, limited amino acid polymorphisms were noted in the MSP-1 gene. Limited amino acid polymorphism was also noted in sequences from autochthonous cases in Italy (Severini et al., 2002), Uzbequistan (Severini et al., 2004), Colombia (Mancilla et al., 1994; Maestre et al., 2004), and Sri Lanka.

Diversity of the MSP-1 gene may impact the ability of the parasite to invade erythrocytes. Regions of this protein, including the region between ICBs 5 and 6, have been shown to be important in attachment to and invasion of erythrocytes (Rodrígues et al., 2002; Espinosa et al., 2003). The MSP-1 protein is also immunogenic (Espinosa et al., 2003, Caro-Aguilar et al., 2002).

Recombination events are believed to occur in the mosquito vector during sexual reproduction. Mosquitoes may become infected with multiple strains by feeding on multiple donors infected with different strains or donors infected with multiple malaria strains.

A comparison of patients treated in the hospital and as outpatients revealed no significant association between MSP-1 sequences and disease severity ($p>0.05$, X^2-test). The hypothesis that emergence of a more virulent strain of *P. vivax* accounted for the increased frequency of hospital admission of infected patients was not supported by analysis of MSP-1 genes.

Recrudescence and relapse infections:

As described in the Methods section, patients in the Alecrim cohort were periodically examined for the presence of malaria parasites in blood smears during treatment with anti-malarial drugs. Successful treatment of *P. vivax* infection included the elimination of parasites from the bloodstream and liver. Recrudescence occurred when blood forms were not eliminated, and then increase during treatment. In contrast, relapse was the reactivation of hypnozoites that were not completely eliminated from the liver (Gilles, 1996; Taylor & Strickland, 2000). Due to limitations in accessing patient information, we could not determine if patients presenting with second time-point infections represented a relapse or a recrudescence. The paired samples from patient K, treated in the hospital, were the only paired samples that presented with identical nucleotide sequences for all of the three genes. All other paired test samples displayed divergence between early and late time-points. The degree of variation, over time, varied among the genes analyzed, less in the 18S SSUrRNA Type A and more in the CSP gene.

The limited polymorphism observed in the 18S SSUrRNA Type A and MSP-1 genes, suggested that the different clones originated from the same progenitors, with the exception of the sample pairs 95A and 95B. Because it was the most conserved of the genes studied, 18S SSUrRNA Type A had the least amount of variation, in comparing paired samples. MSP-1 also displayed limited variability in amino acid sequences, with the exception of the sample pair 95A and B where a number of amino acid substitutions were noted. Seven of the pairs, including the strains from the patient admitted to the hospital were completely identical in 18S SSUrRNA Type A gene sequences. Samples from the patient identified as number 126 had three variable sites, including changes in residues 100 and 117. The two samples from patient 361 were virtually identical, with an A$\rightarrow$T transition at residue 33. When the two new strains were included in the alignment and trees were constructed using distance and parsimony criterion, there was no alteration of the basic topology.

Among the paired CSP amino acid sequences, significant variation was observed in the fifth position of the repeat blocks 1 to 19. There was a highly significant bias towards alanine in sequences from the second infection. Because nonapeptides are known epitopes for CD8+ cytotoxic T-cells and the fifth amino acid is critical in the recognition by T-cell receptors, we hypothesized that changes in position 5 of the repeat block occurred as the result of immunological selection. Accordingly, during the first infection period, parasites with high frequency of aspartic acid at the fifth position induced cellular immunity that selected for the emergence of parasites high frequency of alanine.

Paired sample analyses of *Plasmodium sp*. gene sequences have been conducted by others to evaluate drug efficacy in the treatment of both *P. falciparum* (Cattamanchi et

al., 2003) and *P. vivax* infections (Kirchgatter & del Portillo, 1998). In one study conducted in Uganda, patients were treated for *P. falciparum* infections and the second episodes were classified as reinfections not recrudescence, as patients remained in the transmission zone. In a second study conducted in Brazil, patients acquired malaria in the Amazon region and then returned to their residences in a malaria-free state, São Paulo. New allelic forms were detected in the second infections, and the authors concluded that there might have been two strains involved in the primary infections. In addition, because some of the patients in those studies had previous histories of malaria, relapses from previous infections were also considered possible.

Combined analysis:

It was not possible to demonstrate the evolutionary relationship among our test samples by tests of phylogeny that incorporated sequence data for all three genes tested. The factors that may have limited the power of a combined analysis included small sample size and differences in the mechanisms and extent of variation among the genes. The major variations in the 18S SSUrRNA Type A gene consisted of two common polymorphisms. There were insufficient numbers of additional polymorphisms to permit the construction of a phylogenetic tree representing 18S SSUrRNA Type A sequences with strong bootstrap support. The sources of variation in the CSP gene were the numbers of repeat segments, the A/D polymorphism at position five of the common repeat, a few unusual mutations that occurred coincidently in two to four strains each, and sporadic mutations. These variations permitted the construction of phylogenetic trees with strong bootstrap support. One branch consisting of 15 strains with identical amino acid sequences, including second time-point strains, revealed a strong and meaningful

association. The principal source of variation in the MSP-1 gene was recombination. The importance of recombination in phylogeny was tested by bootstrap analysis, but no gene grouping was associated with the hospitalized patient group. Attempts to construct a consensus using tree from sequences of all three genes resulted in unresolved phylogeny. This finding suggested that the CSP and MSP-1 genes might have evolved somewhat independently. The linkage between the MSP-1 and CSP genes may have been insufficient to permit phylogenetic analysis based on our small sample size. Our inability to derive a single phylogenetic relationship from the *P. vivax* strains tested did not allow a direct test of our principal hypothesis. Particular 18S SSUrRNA Type A and CSP gene sequence characteristics were associated with being in the hospitalized patient group, but were not correlated to each other. This apparent dichotomy could have reflected that one or both associations were chance occurrences. The absence of statistical support for phylogenetic analysis of the SSUrRNA could indicate that the association of the 117:T sequence with hospitalization was serendipitous. The extraordinary frequency of non-synonymous substitutions at the codon for position 5 of the CSP repeat blocks, the very high frequency of alanine at this position in the hospitalized patients, the potential significance of alanine for aspartic acid substitutions in general and its specific potential significance at the central residue of a CTL epitope. And the high frequency of alanine for aspartate substitutions in paired samples all supported our hypothesis that the A/D polymorphism in the CSP repeat blocks may have been related to pathogenicity. In any case, additional genetic analyses are needed to determine if a new variant of *P. vivax* might be associated with an increased propensity to cause severe thrombocytopenia and disease.

5. Study limitations:

The limited sample size and the broad case definition used to define severe disease and admit patients to the hospital may have limited our ability to find a difference between treatment groups. In addition, it is possible that a selection bias was introduced when patients from the original cohort were selected. There was no rigorous protocol for the selection of participants and the sample was convenience-based.

The selection of marker genes for the phylogenetic analysis of *P. vivax* was limited to what is available in Gene Bank and is described in the literature. We were also unable to amplify the parasite DNA of historical blood smears.

6. Future studies:

To further investigate the association of genetic profile and disease manifestation we suggest two types of studies, one directed to the phylogenetic analysis and other directed to the pathogenic hypothesis.

Future phylogenetic studies of *P. vivax* population in Amazonas State could include analysis of longer segments of the SSUrRNA Type A gene, use of other MSP sequences, such as MSP-3α, or a different block of the MSP-1 gene. In addition, we could use a different genetic marker, such as apical membrane antigen-1 (AMA-1). Use of high fidelity polymerases and direct sequence of PCR products would be used to minimize the introduction of artifacts.

There should be four goals in the genotyping study: evaluation of whether the 117:T mutation in the 18S SSUrRNA Type A gene is associated with pathogenicity;

evaluation of whether the D/A frequency in the fifth position residues is associated with pathogenicity, and evaluation of whether this association reflects common ancestry pathogenic strains or is independent of ancestry. Determination of whether there is linkage between the 117T and D/A polymorphisms; determination of whether there is a genotype associated with pathogenicity. The latter determination will require sampling a gene in which variation is mostly or exclusively through mutation rather than recombination and for which there will be enough sequence variation by virtue of numbers of samples tested and length of the gene assessed to permit definitive definition of phylogeny.

To test the pathogenicity hypothesis we could develop a prospective follow-up study and evaluate T-cell epitopes in the Amazon region population. A randomized selection of patients and a more restrictive case definition would be established. Cases would be defined as patients presenting platelet levels equal to or below 50,000/mm^3. HLA type would be determined, as there are differential responses for epitopes based on the genetic make up of the population. During follow-up patients would have sequential blood collections to determine the evolution and clearing of parasitemia, determined by blood smear and PCR analysis. An extended follow-up would be included to determine the presence of relapses and/or new infections; this information would be used to evaluate the in patient evolution of the parasites.

The study should be powered to permit accomplishment of the goals. For that we would take into consideration the number of malaria cases and admissions due to *P. vivax*; number of patients with low platelet levels, determined by review of the malaria

charts at FMT-Am; and, frequency of mutations in the genes selected for studies, determined by a pilot study and review of published sequences.

7. Conclusions:

Our data demonstrates that malaria continues to be a health issue in the Amazon Region of Brazil, with evidence of an epidemic that continues to emerge, and disease caused by *Plasmodium vivax* now posing a significant and increasing threat to the health of the population.

Genetic diversity in the *P. vivax* population from Amazonas resulted from synonymous and non-synonymous substitutions, insertions, deletions and recombination. The diversity observed in the CSP gene appeared to be generated by selection during human infection, while the diversity observed in the MSP-1 gene was originated by recombination during the sexual reproduction perhaps, in the mosquito host.

This retrospective study was unable to demonstrate that a particular strain of *Plasmodium vivax* is responsible for severe disease requiring hospitalization. Due to continued emergence of the *P. vivax* epidemic in Brazil a prospective study of current severe cases is warranted.

Variation of genes encoding antigens that are vaccine development targets is extensive and more study of the relationship between genetic and antigenic variation is needed.

8. Annex:

8.1 Paup Scripts:

Distance based tree search – Neighbor-Joining:

```
#nexus
log file="name".txt;
set criterion=distance;
dset     distance=p rates=equal objective=me;
nj       brlens=yes breakties=random;
describetrees all/       plot=phylogram brlens=yes root=outgroup outroot=monophyl;
savetrees        brlens=yes file="name".trees;
dset     distance=JC rates=equal objective=me;
nj       brlens=yes breakties=random ;
describetrees all/       plot=phylogram brlens=yes root=outgroup outroot=monophyl;
savetrees        brlens=yes file="name".trees;
dset     distance=tamnei rates=equal objective=me;
nj       brlens=yes breakties=random;
describetrees all/       plot=phylogram brlens=yes root=outgroup outroot=monophyl;
savetrees        brlens=yes file="name".trees;
dset     distance=hky85 rates=equal objective=me;
nj       brlens=yes breakties=random;
describetrees all/       plot=phylogram brlens=yes root=outgroup outroot=monophyl;
savetrees        brlens=yes file="name".trees;
```

dset distance=gtr rates=equal objective=me;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

det distance=logdet rates=equal objective=me;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=p rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=jc rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=tamnei rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=hky85 rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=gtr rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

dset distance=logdet rates=equal objective=lsfit;

nj brlens=yes breakties=random;

describetrees all/ plot=phylogram brlens=yes root=outgroup outroot=monophyl;

savetrees brlens=yes file="*name*".trees;

To get trees from file and construct a consensus tree:

#nexus

Gettrees File = "*name*".trees From=1 to=1 Mode=7;

Contree all/ majrule=yes strict=no indices=yes grpfreq=yes treefile="*name*".trees;

To construct a tree with Neighbor-Joining and perform Bootstrap analysis:

#nexus

log file="nane".txt;

set criterion=distance; dset distance=hky85 objective=me;

nj brlens=yes breakties=random; describetrees all/ plot=phylogram brlens=yes

root=outgroup outroot=monophyl; savetrees brlens=yes file="nane".trees;

bootstrap treefile="nane".trees nreps=1000 conlevel=50 grpfreq=yes

cutoffpct=50 search=nj nreps=1000;

savetrees file="nane".trees format=phylip brlens=yes savebootp=nodelabels

maxdecimals=1 from=1 to=1;

Parsimony based tree search - Heuristic Search under parsimony

Considering Gaps a 5th base:

#Nexus

Log file="*name*".txt;

Set Criterion=parsimony Storebrlens=yes Storetreewts=yes;

Pset Gapmode=newstate Stepmatrix=allstates;

Hsearch Swap=tbr Addseq=random;

Savetrees File="*name*".trees Format=nexus Brlens=yes;

Clear;

Gettrees File="*name*".trees From=1 to=5000 Storebrlens=yes;

Contree all/ majrule=yes strict=no Indices=yes grpfreq=yes Treefile="*name*".trees;

Considering Gaps as missing data:

Change the following option

Gapmode=missing

Bootstrap analysis using Heuristic Search under parsimony:

#Nexus

Set Criterion=parsimony;

Log file="*name*".txt;

Bootstrap Treefile="*name*".trees Nreps=1000 Conlevel = 50 Grpfreq = yes Cutoffpct = 5 Search = heuristic nreps = 1000;

Savetrees File="*name*".trees Format=phylip brlens=yes savebootp=nodelabels maxdecimals=1 from=1 to=1;

8.2 Reagents and Kits Used in the Protocols:

From Gentra Systems (Minneapolis, Mn):

Puregene™ DNA Purification System

- Blood Kit: D-5500
- Purescript® Glycogen Solution (20mg/mL): R-5010
- Proteinase K Solution (20mg/mL)

From The Warner-Graham Company (Cokeysville-MD):

Ethyl Alcohol U.S.P. 200 proof

From J.T.Parker (Philipsurg-NJ):

Isoporopanol U.S.P.: 9080-01

From QB Quality Biological, Inc. (Gaithersburg-MD):

DEPC treated water: 351-068-061

From QIGEN Inc. (Valencia-CA):

QIAquick PCR Purification kit: 28106

QIAquick Gel Extraction Kit: 28706

HotStarTaq® DNA Polymerase Kit (1000 Units): 203205

QIAamp DNA mini kit: 51306

QIAamp DNA Blood Mini Kit: 51106

QIAprep Spin Miniprep Kit: 27106

ProofStart™ DNA Polymerase (500 Units): 202205

From Applied Biosystems (Foster City-CA):

GeneAmp® 10mM dNTP mix with dTTP: N808-0260

GeneAmp® XL PCR Kit: N808-0192

From Invitrogen (Carlsbad, CA):

MAX Efficiency® DH5α™ Competent Cells: 18258-012

TOPO TA Cloning® Kit for Sequencing: K4575-01

From SIGMA (Saint Louis-Mo):

DNA ladder, DIRECTLOAD™, 100 bp: D-3687

Deoxynucleotide (dNTP) mix, 10mM Solution: D-7295

Taq Superpack™ DNA Polymerase without $MgCl_2$: D-5813

From New England BioLabs® Inc. (Beverly-MA):

Restriction Enzymes: Kpn I (R0142L), Sac I (R0156L), Pvu II (R0151S), BstX I (R0113S), Bcl I (R0527S)

From Edge BioSystems (Gaithersburg-MD):

Performa® DTR Gel Filtration Cartridges: 42453

From Stratagene (La Jolla-CA):

PCR-Script™ Amp Clining Kit: 211188

From Gibco BRL® (Gaithersburg-MD):

Ethidium Bromide (10mg/mL solution): 15585-011

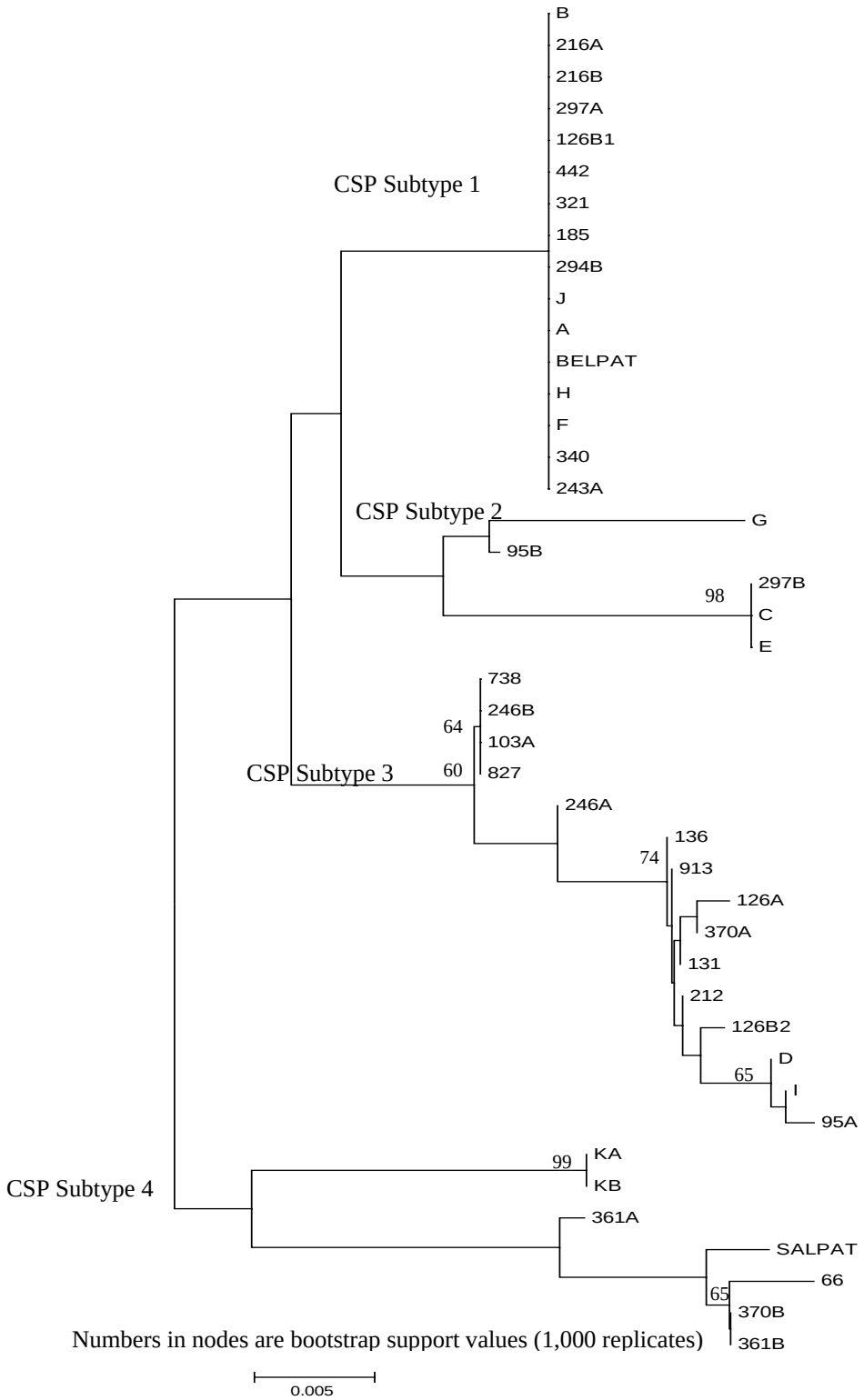
8.3 Equipments Used in the Protocols:

PTC-200 thermocycler (MJ Research Inc., Waltham, MA)

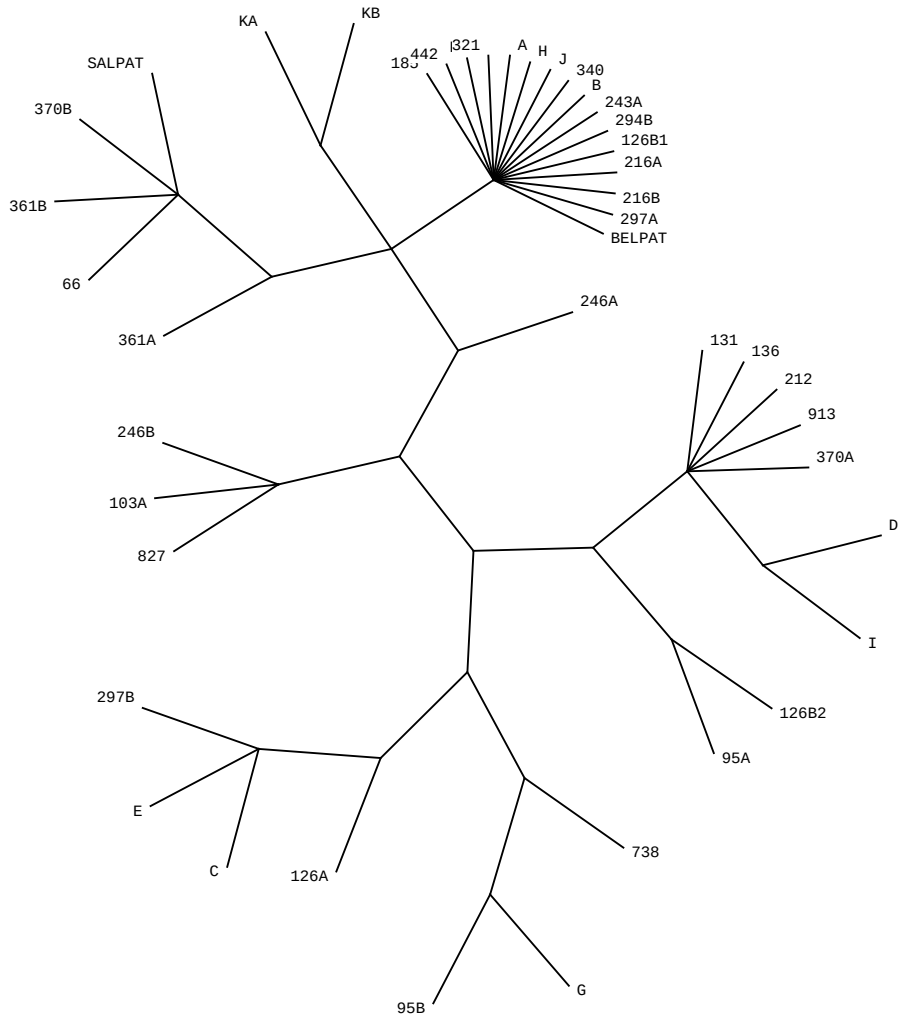
Eppendorf microcentrifuge 5415 D (Westbury, NY)

8.4 Annex Figures:

Annex Figure 1: Neighbor-Joining tree showing inferred phylogeny of CSP amino acid sequence of strains from Manaus and references strains Salvador I (SALPAT) and Belém (BELPAT). Tree constructed using the distance method, amino (p-distance).

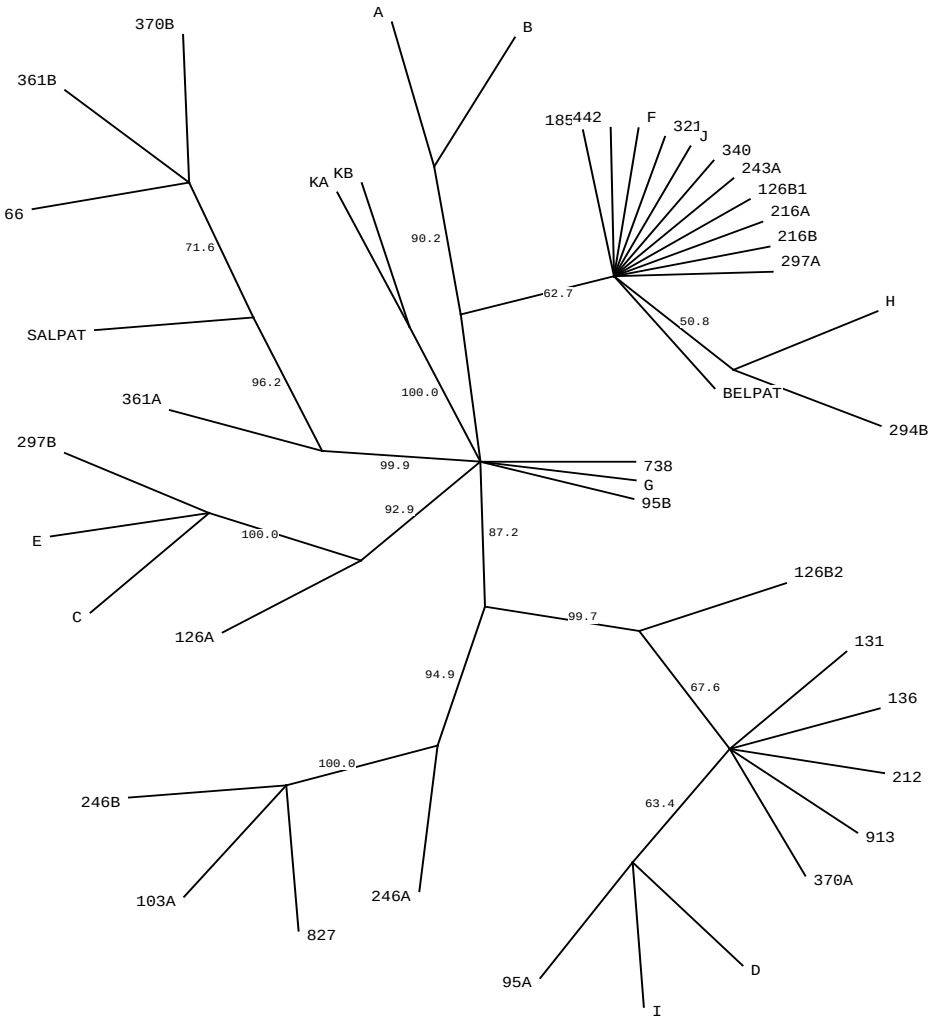


Annex Figure 2: Fifty percent majority rule consensus of 63 trees found by heuristic search showing inferred phylogeny of CSP nucleotide sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELPAT).

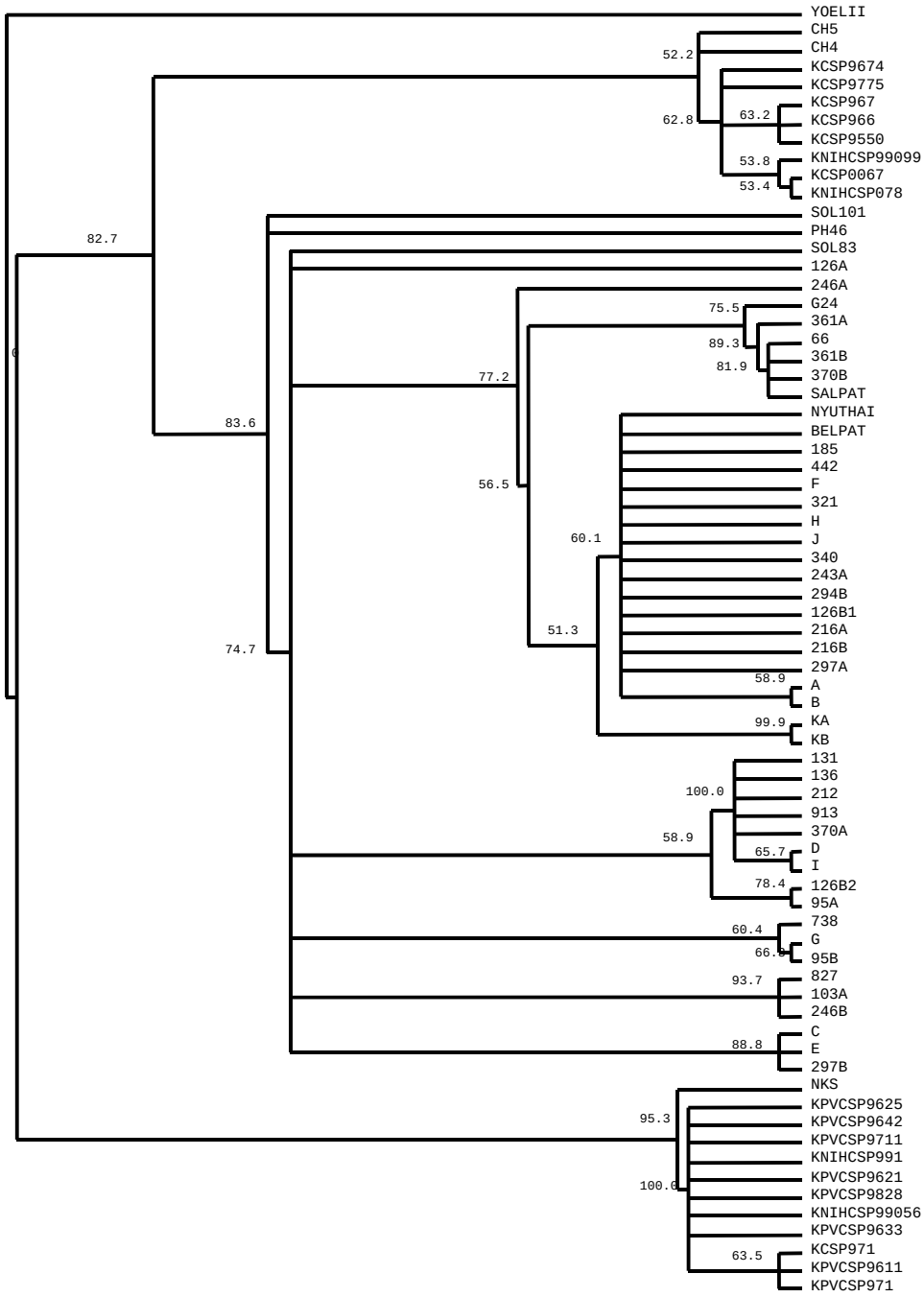


Nucleotide sequence: 848 total characters, 703 constant, 145 variables, 9 parsimony non-informative and 136 parsimony-informative.
Heuristic search under the optimality criterion of Parsimony; gaps in the nucleotide alignment were treated as 5th base, starting trees were obtained by stepwise addition, sequences were added at random and tree-bisection-reconnection was the branch-swapping algorithm.

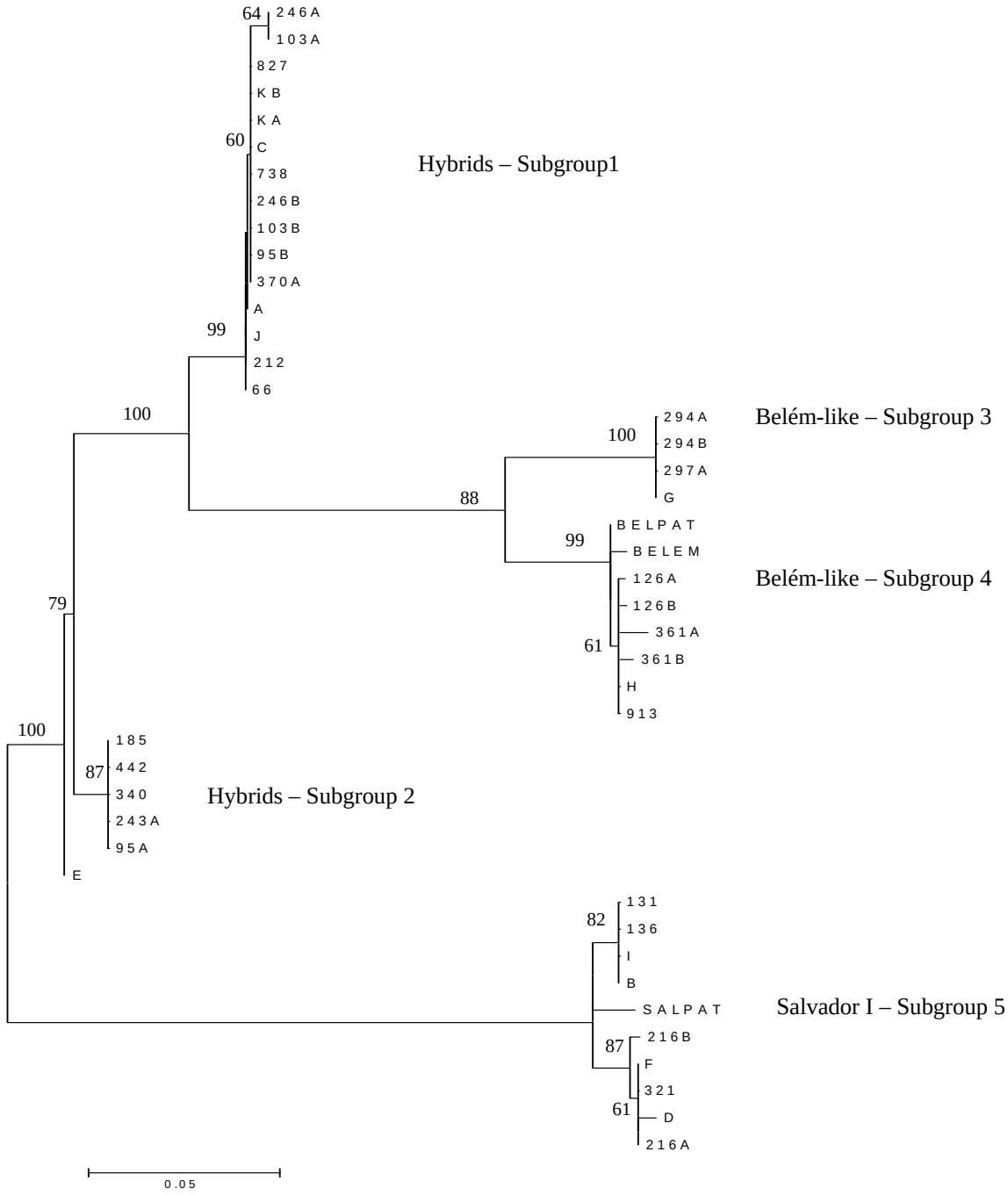
Annex Figure 3: Bootstrap 50% majority-rule consensus tree of CSP gene nucleotide sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELPAT). Optimality criterion set to distance (minimum evolution) measured by the HKY85 method. Bootstrap analysis performed with 1,000 replicates.



Annex Figure 4: Bootstrap 50% majority-rule consensus tree of CSP nucleotide sequence of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELPAT) and sequences from Gene Bank. Bootstrap support value in each node.

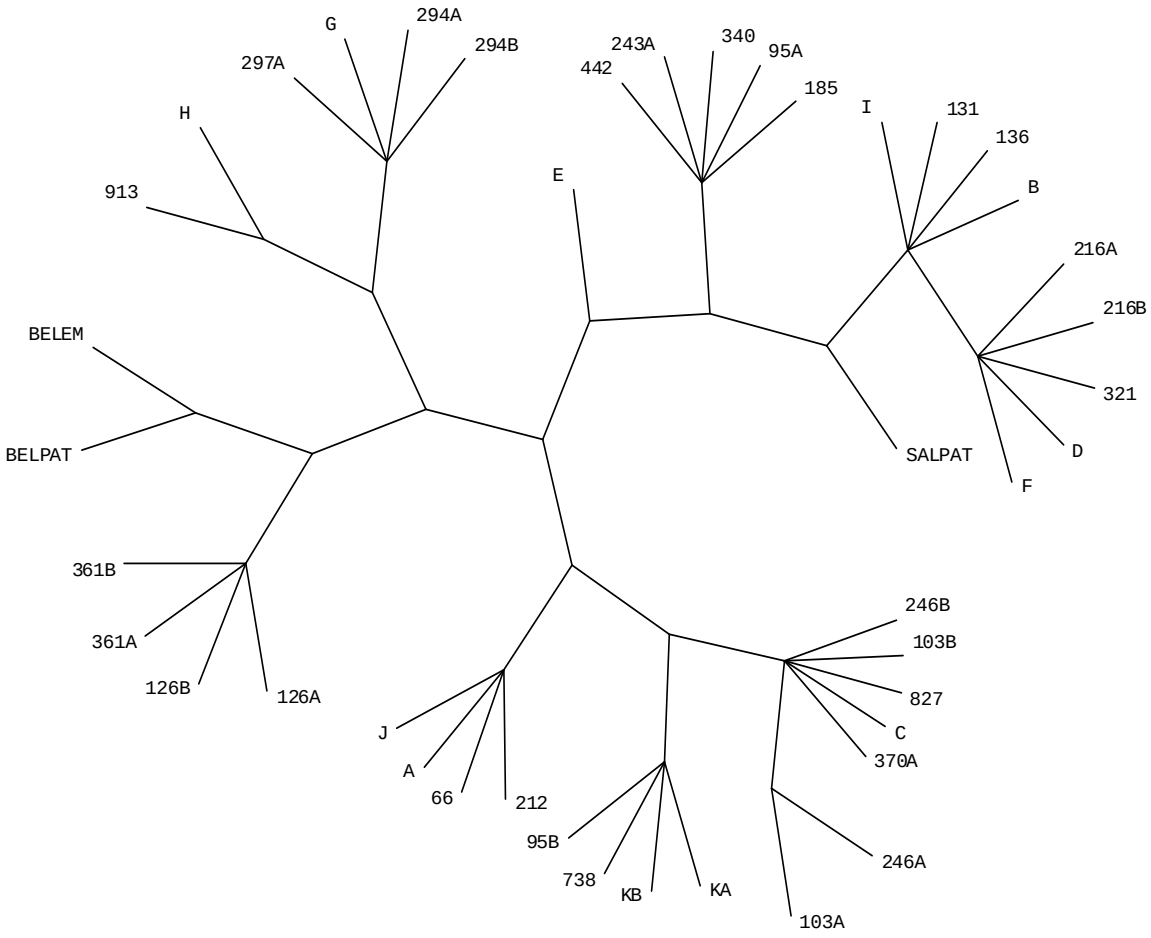


Annex Figure 5: Neighbor-Joining tree showing inferred phylogeny of MSP-1 amino acid sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT). Tree constructed using the distance method, amino (p-distance).



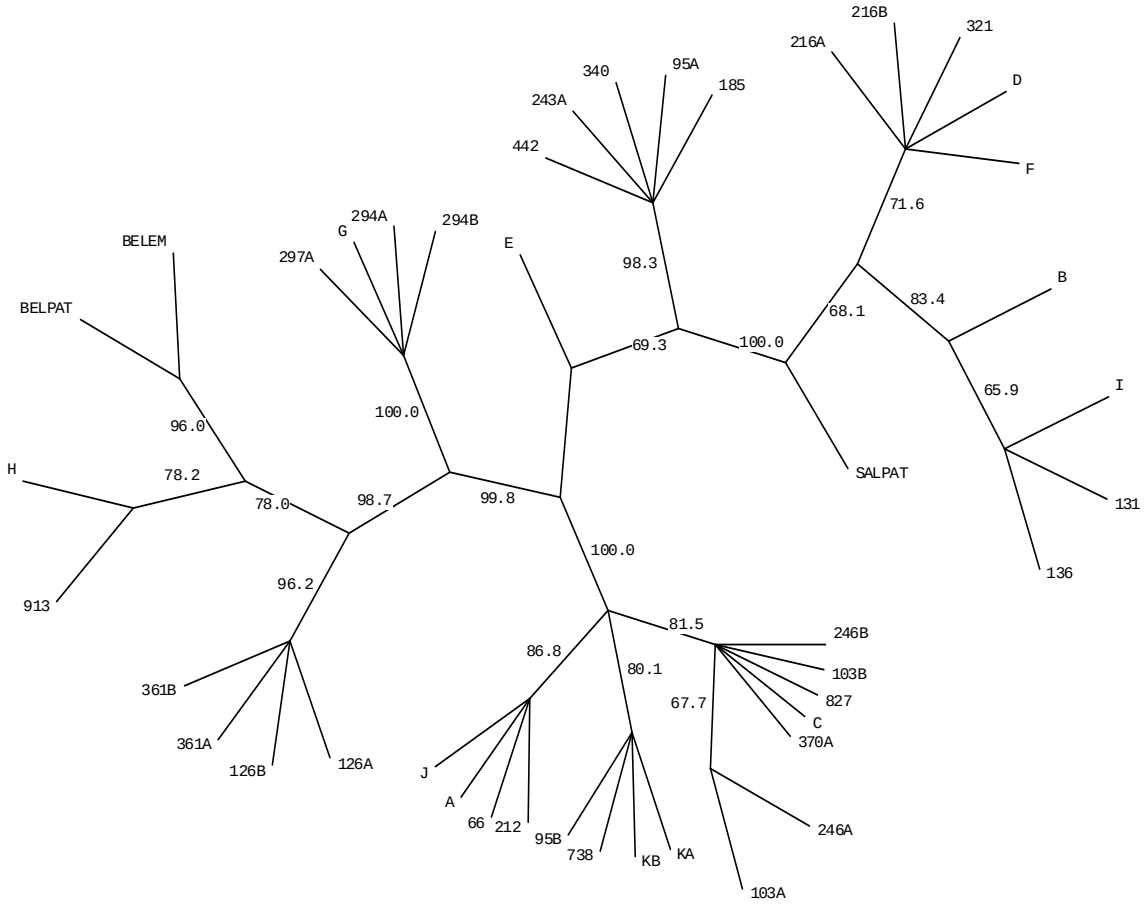
Numbers in nodes are bootstrap support values (1,000 replicates)

Annex Figure 6: Fifty percent majority rule consensus of 16 trees found by heuristic search showing inferred phylogeny of MSP-1 nucleotide sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT).

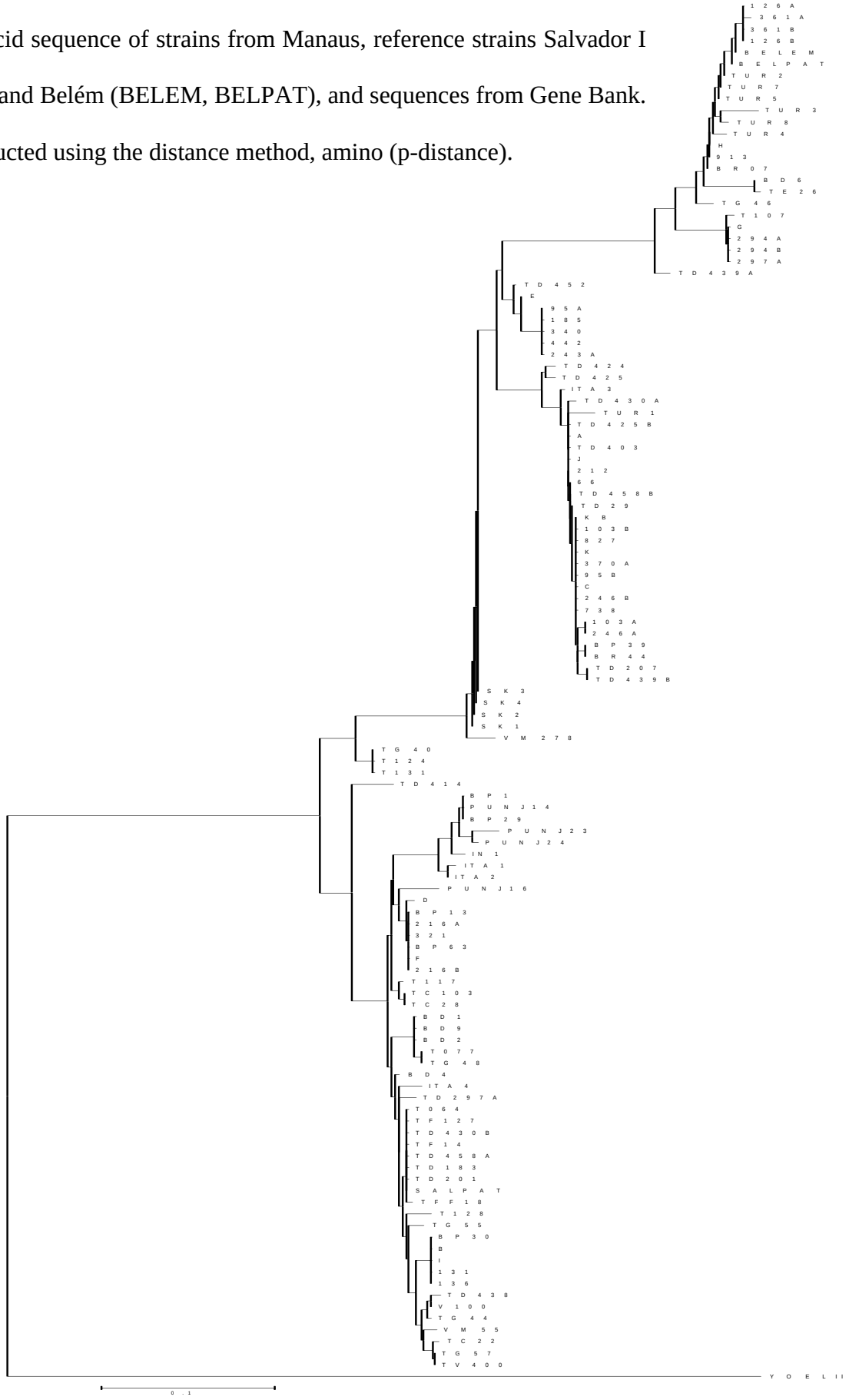


Nucleotide sequence: 668 total characters, 439 constant, 229 variables, 7 parsimony non-informative and 222 parsimony-informative.
Heuristic search under the optimality criterion of Parsimony; gaps in the nucleotide alignment were treated as 5th base, starting trees were obtained by stepwise addition, sequences were added at random and tree-bisection-reconnection was the branch-swapping algorithm.

Annex Figure 7: Bootstrap 50% majority-rule consensus tree of MSP-1 gene nucleotide sequence of strains from Manaus and reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT). Optimality criterion set to distance (Minimum evolution) measured by the HKY85 method. Bootstrap analysis performed with 1,000 replicates.



Annex Figure 8: Neighbor-Joining tree showing inferred phylogeny of MSP –1 amino acid sequence of strains from Manaus, reference strains Salvador I (SALPAT) and Belém (BELEM, BELPAT), and sequences from Gene Bank. Tree constructed using the distance method, amino (p-distance).



8.5 Annex Alignments:

Alignment 1: 18S SSUrRNA Type A gene, nucleotide sequence alignment of strains from Manaus, references Salvador (SALPAT) and Belém (BELPAT) and sequences retrieved from Gene Bank. Identical nucleotides represented by dots, gaps represented by dashes.

```
ELSAL          TCTGGTTAAT TCCGATAACG AACGAGATCT TAACCTGCTA ATTAGCGGCA  [ 50]
BELPAT         .......... .......... .......... .......... ..........  [ 50]
SALPAT         .......... .......... .......... .......... ..........  [ 50]
PVTHAI         .......... .......... .......... .......... ..........  [ 50]
SALI           .......... .......... .......... .......... ..........  [ 50]
CYNOMOLGY      .......... .......... .......... .......... ..........  [ 50]
COLOMBIA       .......... .......... .......... .......... ..........  [ 50]
I - ADM        .......... .......... .......... .......... ..........  [ 50]
E - ADM        .......... .......... .......... .......... ..........  [ 50]
H - ADM        .......... .......... .......... .......... ..........  [ 50]
D - ADM        .......... .......... .......... .......... ..........  [ 50]
F - ADM        .......... .......... .......... .......... ..........  [ 50]
G - ADM        .......... .......... .......... .......... ..........  [ 50]
J - ADM        .......... .......... .......... .......... ..........  [ 50]
KA - ADM       .......... .......... .......... .......... ..........  [ 50]
KB - ADM       .......... .......... .......... .......... ..........  [ 50]
A - ADM        .......... .......... .......... .......... ..........  [ 50]
C - ADM        .......... .......... .......... .......... ..........  [ 50]
B - ADM        .......... .......... .......... .......... ..........  [ 50]
136 - NOREL    .......... .......... .......... .......... ..........  [ 50]
131 - NOREL    .......... .......... .......... .......... ..........  [ 50]
212 - NOREL    .......... .......... .......... .......... ..........  [ 50]
66 - NOREL     .......... .......... .......... .......... ..........  [ 50]
185 - NOREL    .......... .......... .......... .......... ..........  [ 50]
340 - NOREL    .......... .......... .......... .......... ..........  [ 50]
913 - NOREL    .......... .......... .......... .......... ..........  [ 50]
442 - NOREL    .......... .......... .......... .......... ..........  [ 50]
738 - NOREL    .......... .......... .......... .......... ..........  [ 50]
321 - NOREL    .......... .......... .......... .......... ..........  [ 50]
827 - NOREL    .......... .......... .......... .......... ..........  [ 50]
243A - REL     .......... .......... .......... .......... ..........  [ 50]
361A - REL     .......... .......... .......... ..T....... ..........  [ 50]
126A - REL     .......... .......... .......... .......... ..........  [ 50]
294A - REL     .......... .......... .......... .......... ..........  [ 50]
294B - REL     .......... .......... .......... .......... ..........  [ 50]
297A - REL     .......... .......... .......... .......... ..........  [ 50]
126B - REL     .......... .......... .......... .......... ..........  [ 50]
246A - REL     .......... .......... .......... .......... ..........  [ 50]
246B - REL     .......... .......... .......... .......... ..........  [ 50]
103B - REL     .......... .......... .......... .......... ..........  [ 50]
95B - REL      .......... .......... .......... .......... ..........  [ 50]
103A - REL     .......... .......... .......... .......... ..........  [ 50]
370A - REL     .......... .......... .......... .......... ..........  [ 50]
216B - REL     .......... .......... .......... .......... ..........  [ 50]
216A - REL     .......... .......... .......... .......... ..........  [ 50]
361B - REL     .......... .......... .......... .......... ..........  [ 50]
370B - REL     .......... .......... .......... .......... ..........  [ 50]
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```
95A - REL     .......... .......... .......... .......... ..........  [ 50]
```

Alignment 1 continuing

```
ELSAL         AATACGACAT ATTCTTACGT GGGACTGAAT TCGGTTGATT TGCTTACTTT  [100]
BELPAT        .......T.. .......... .......... .......... ..........  [100]
SALPAT        .......T.. .......... .......... .......... ..........  [100]
PVTHAI        .......T.. .......... .......... .......... ..........  [100]
SALI          .......T.. .......... .......... .......... .........C  [100]
CYNOMOLGY     .......T.. .......TA. ....T..... A......... ......T...  [100]
COLOMBIA      .......T.. .......... .......... .......... ..........  [100]
I - ADM       .......T.. .......... .......... .......... ..........  [100]
E - ADM       .......T.. .......... .......... .......... ..........  [100]
H - ADM       .......T.. .......... .......... .......... ..........  [100]
D - ADM       .......T.. .......... .......... .......... .........C  [100]
F - ADM       .......T.. .......... .......... .......... .........C  [100]
G - ADM       .......T.. .......... .......... .......... .........C  [100]
J - ADM       .......T.. .......... .......... .......... ..........  [100]
KA - ADM      .......T.. .......... .......... .......... ..........  [100]
KB - ADM      .......T.. .......... .......... .......... ..........  [100]
A - ADM       .......T.. .......... .......... .......... .........C  [100]
C - ADM       .......T.. .......... ....T..... .......... .........C  [100]
B - ADM       .......T.. .......... .......... .......... ..........  [100]
136 - NOREL   .......T.. .......... .......... .......... ..........  [100]
131 - NOREL   .......T.. .......... .......... .......... ..........  [100]
212 - NOREL   .......T.. .......... .......... .......... .........C  [100]
66 - NOREL    .......T.. .......... .......... .......... .........C  [100]
185 - NOREL   .......T.. .......... .......... .......... ..........  [100]
340 - NOREL   .......T.. .......... .......... .......... ..........  [100]
913 - NOREL   .......T.. .......... .......... .......... ..........  [100]
442 - NOREL   .......T.. .......... .......... .......... ..........  [100]
738 - NOREL   .......... .......... .......... .......... ..........  [100]
321 - NOREL   .......T.. .......... .......... .......... ..........  [100]
827 - NOREL   .......... .......... .......... .......... ..........  [100]
243A - REL    .......T.. .......... ....T..... .......... .........C  [100]
361A - REL    .......T.. .......... .......... .......... ..........  [100]
126A - REL    .......T.. ......G... .......... .......... ..........  [100]
294A - REL    .......T.. .......... .......... .......... .........C  [100]
294B - REL    .......T.. .......... .......... .......... .........C  [100]
297A - REL    .......T.. .......... .......... .......... .........C  [100]
126B - REL    .......T.. .......... .......... .......... .........C  [100]
246A - REL    .......T.. .......... .......... .......... ..........  [100]
246B - REL    .......T.. .......... .......... .......... ..........  [100]
103B - REL    .......T.. .......... .......... .......... ..........  [100]
95B - REL     .......T.. .......... .......... .......... ..........  [100]
103A - REL    .......T.. .......... .......... .......... ..........  [100]
370A - REL    .......T.. .......... .......... .......... ..........  [100]
216B - REL    .......T.. .......... .......... .......... ..........  [100]
216A - REL    .......T.. .......... .......... .......... ..........  [100]
361B - REL    .......T.. .......... .......... .......... ..........  [100]
370B - REL    .......T.. .......... .......... .......... ..........  [100]
95A - REL     .......T.. .......... .......... .......... ..........  [100]
```

Alignment 1 continuing

```
ELSAL          GAAGAAAATA TTGGGATACG TAACAGTTTC CCTTTCCCTT TTCTACTTAG  [150]
BELPAT         .......... ......A... .......... .......... ..........  [150]
SALPAT         .......... .......... .......... .......... ..........  [150]
PVTHAI         .......... ......A... .......... .......... ..........  [150]
SALI           .......... .......... .......... .......... ..........  [150]
CYNOMOLGY      .......... .......G.. ...-...G.. .......... .........A  [150]
COLOMBIA       .......... ......A... .......... .......... ..........  [150]
I - ADM        .......... .......... .......... .......... ..........  [150]
E - ADM        .......... .......... .......... .......... ..........  [150]
H - ADM        .......... .......... .......... .......... ..........  [150]
D - ADM        .......... .......... .......... .......... ..........  [150]
F - ADM        .......... .......... .......... .......... ..........  [150]
G - ADM        .......... .......... .......... .......... ..........  [150]
J - ADM        .......... ........T. .......... .......... ..........  [150]
KA - ADM       .......... .......... .......... .......... ..........  [150]
KB - ADM       .......... .......... .......... .......... ..........  [150]
A - ADM        .......... ........T. .......... .......... ..........  [150]
C - ADM        .......... .......... ...A...... .......... ..........  [150]
B - ADM        .......... ......A... .......... .......... ..........  [150]
136 - NOREL    .......... ......A... .......... .......... ..........  [150]
131 - NOREL    .......... ......A... .......... .......... ..........  [150]
212 - NOREL    .......... .......... .......... .......... ..........  [150]
66 - NOREL     .......... ......AG.. .......... .......... ..........  [150]
185 - NOREL    .......... ......A... .......... .......... ..........  [150]
340 - NOREL    .......... ......A... .......... .......... ..........  [150]
913 - NOREL    .......... ......A... .......... .......... ..........  [150]
442 - NOREL    .......... ......A... .......... .......... ..........  [150]
738 - NOREL    .......... ......A... .......... .......... ..........  [150]
321 - NOREL    .......... ......A... .......... .......... ..........  [150]
827 - NOREL    .......... .......... .......... .......... ..........  [150]
243A - REL     .......... .......... .......... .......... ..........  [150]
361A - REL     .......... ......A... .......... .......... ..........  [150]
126A - REL     .......... ......A... .......... .......... ..........  [150]
294A - REL     .......... .......... .......... .......... ..........  [150]
294B - REL     .......... .......... .......... .......... ..........  [150]
297A - REL     .......... .......... .......... .......... ..........  [150]
126B - REL     .......... .......... .......... .......... ..........  [150]
246A - REL     .......... .......... .......... .......... ..........  [150]
246B - REL     .......... .......... .......... .......... ..........  [150]
103B - REL     .......... .......... .......... .......... ..........  [150]
95B - REL      .......... ......A... .......... .......... ..........  [150]
103A - REL     .......... .......... .......... .......... ..........  [150]
370A - REL     .......... .......... .......... .......... ..........  [150]
216B - REL     .......... ......A... .......... .......... ..........  [150]
216A - REL     .......... ......A... .......... .......... ..........  [150]
361B - REL     .......... ......A... .......... .......... ..........  [150]
370B - REL     .......... .......... .......... .......... ..........  [150]
95A - REL      .......... ......A... .......... .......... ..........  [150]
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Alignment 1 continuing

```
ELSAL          TTCGCTTTTC ATACTGTTTC TTTTTCGCGT AAGAATGTAT TTGCTTGATT  [200]
BELPAT         .......... .......... .......... .......... ..........  [200]
SALPAT         .......... .......... .......... .......... ..........  [200]
PVTHAI         .......... .......... .......... .......... ..........  [200]
SALI           .......... .......... .......... .......... ..........  [200]
CYNOMOLGY      .......A.. .......... .......T.. .......... ..........  [200]
COLOMBIA       .......... .......... .......... .......... ..........  [200]
I - ADM        .......... .......... .......... .......... ..........  [200]
E - ADM        .......... .......... .......... .......... ..........  [200]
H - ADM        .......... .......... .......... .......... ..........  [200]
D - ADM        .......... .......... .......... .......... ..........  [200]
F - ADM        .......... .......... .......... .......... ..........  [200]
G - ADM        .......... .......... .......... .......... ..........  [200]
J - ADM        .......... .......... .......... .......... ..........  [200]
KA - ADM       .......... .......... .......... .......... ..........  [200]
KB - ADM       .......... .......... .......... .......... ..........  [200]
A - ADM        .......... .......... .......... .......... ..........  [200]
C - ADM        .......... .......... .......... .......... ..........  [200]
B - ADM        .......... .......... .......... .......... ..........  [200]
136 - NOREL    .......... .......... .......... .......... ..........  [200]
131 - NOREL    .......... .......... .......... .......... ..........  [200]
212 - NOREL    .......... .......... .......... .......... ..........  [200]
66 - NOREL     .......... .......... .......... .......... ..........  [200]
185 - NOREL    .......... .......... .......... .......... ..........  [200]
340 - NOREL    .......... .......... .......... .......... ..........  [200]
913 - NOREL    .......... .......... .......... .......... ..........  [200]
442 - NOREL    .......... .......... .......... .......... ..........  [200]
738 - NOREL    .......... .......... .......... .......... ..........  [200]
321 - NOREL    .......... .......... .......... .......... ..........  [200]
827 - NOREL    .......... .......... .......... .......... ..........  [200]
243A - REL     .......... .......... .......... .......... ..........  [200]
361A - REL     .......... .......... .......... .......... ..........  [200]
126A - REL     .......... .......... .......... .......... ..........  [200]
294A - REL     .......... .......... .......... .......... ..........  [200]
294B - REL     .......... .......... .......... .......... ..........  [200]
297A - REL     .......... .......... .......... .......... ..........  [200]
126B - REL     .......... .......... .......... .......... ..........  [200]
246A - REL     .......... .......... .......... .......... ..........  [200]
246B - REL     .......... .......... .......... .......... ..........  [200]
103B - REL     .......... .......... .......... .......... ..........  [200]
95B - REL      .......... .......... .......... .......... ..........  [200]
103A - REL     .......... .......... .......... .......... ..........  [200]
370A - REL     .......... .......... .......... .......... ..........  [200]
216B - REL     .......... .......... .......... .......... ..........  [200]
216A - REL     .......... .......... .......... .......... ..........  [200]
361B - REL     .......... .......... .......... .......... ..........  [200]
370B - REL     .......... .......... .......... .......... ..........  [200]
95A - REL      .......... .......... .......... .......... ..........  [200]
```

Alignment 1 continuing

```
ELSAL          GTAAAGCTTC TTAGAGGAAC GATGTGTGTC TAACACAAGG AAGTTTAAGG  [250]
BELPAT         .......... .......... .......... .......... ..........  [250]
SALPAT         .......... .......... .......... .......... ..........  [250]
PVTHAI         .......... .......... .......... .......... ..........  [250]
SALI           .......... .......... ........C. .......... ..........  [250]
CYNOMOLGY      .......... .......... .......... .......... ..........  [250]
COLOMBIA       .......... .......... .......... .......... ..........  [250]
I - ADM        .......... .......... .......... .......... ..........  [250]
E - ADM        .......... .......... .......... .......... ..........  [250]
H - ADM        .......... .......... .......... .......... ..........  [250]
D - ADM        .......... .......... .......... .......... ..........  [250]
F - ADM        .......... .......... .......... .......... ..........  [250]
G - ADM        .......... .......... .......... .......... ..........  [250]
J - ADM        .......... .......... .......... .......... ..........  [250]
KA - ADM       .......... .......... .......... .......... ..........  [250]
KB - ADM       .......... .......... .......... .......... ..........  [250]
A - ADM        .......... .......... .......... .......... ..........  [250]
C - ADM        .......... .......... .......... .......... ..........  [250]
B - ADM        .......... .......... .......... .......... ..........  [250]
136 - NOREL    .......... .......... .......... .......... ..........  [250]
131 - NOREL    .......... .......... .......... .......... ..........  [250]
212 - NOREL    .......... .......... .......... .......... ..........  [250]
66 - NOREL     .......... .......... .......... .......... ..........  [250]
185 - NOREL    .......... .......... .......... .......... ..........  [250]
340 - NOREL    .......... .......... .......... .......... ..........  [250]
913 - NOREL    .......... .......... .......... .......... ..........  [250]
442 - NOREL    .......... .......... .......... .......... ..........  [250]
738 - NOREL    .......... .......... .......... .......... ..........  [250]
321 - NOREL    .......... .......... .......... .......... ..........  [250]
827 - NOREL    .......... .......... .......... .......... ..........  [250]
243A - REL     .......... .......... .......... .......... ..........  [250]
361A - REL     .......... .......... .......... .......... ..........  [250]
126A - REL     .......... .......... .......... .......... ..........  [250]
294A - REL     .......... .......... .......... .......... ..........  [250]
294B - REL     .......... .......... .......... .......... ..........  [250]
297A - REL     .......... .......... .......... .......... ..........  [250]
126B - REL     .......... .......... .......... .......... ..........  [250]
246A - REL     .......... .......... .......... .......... ..........  [250]
246B - REL     .......... .......... .......... .......... ..........  [250]
103B - REL     .......... .......... .......... .......... ..........  [250]
95B - REL      .......... .......... .......... .......... ..........  [250]
103A - REL     .......... .......... .......... .......... ..........  [250]
370A - REL     .......... .......... .......... .......... ..........  [250]
216B - REL     .......... .......... .......... .......... ..........  [250]
216A - REL     .......... .......... .......... .......... ..........  [250]
361B - REL     .......... .......... .......... .......... ..........  [250]
370B - REL     .......... .......... .......... .......... ..........  [250]
95A - REL      .......... .......... .......... .......... ..........  [250]
```

Alignment 1 continuing

```
ELSAL          CAACAACAGG TCTGTGATGT CCTTAGATGA ACTAGGCTGC ACGCGTGCTA  [300]
BELPAT         .......... .......... .......... .......... ..........  [300]
SALPAT         .......... .......... .......... .......... ..........  [300]
PVTHAI         .......... .......... .......... .......... ..........  [300]
SALI           .......... .......... .......... .......... ..........  [300]
CYNOMOLGY      .......... .......... .......... .......... ..........  [300]
COLOMBIA       .......... .......... .......... .......... ..........  [300]
I - ADM        .......... .......... .......... .......... ..........  [300]
E - ADM        .......... .......... .......... .......... ..........  [300]
H - ADM        .......... .......... .......... .......... ..........  [300]
D - ADM        .......... .......... .......... .......... ..........  [300]
F - ADM        .......... .......... .......... .......... ..........  [300]
G - ADM        .......... .......... .......... .......... ..........  [300]
J - ADM        .......... .......... .......... .......... ..........  [300]
KA - ADM       .......... .......... .......... .......... ..........  [300]
KB - ADM       .......... .......... .......... .......... ..........  [300]
A - ADM        .......... .......... .......... .......... ..........  [300]
C - ADM        .......... .......... .......... .......... ..........  [300]
B - ADM        .......... .......... .......... .......... ..........  [300]
136 - NOREL    .......... .......... .......... .......... ..........  [300]
131 - NOREL    .......... .......... .......... .......... ..........  [300]
212 - NOREL    .......... .......... .......... .......... ..........  [300]
66 - NOREL     .......... .......... .......... .......... ..........  [300]
185 - NOREL    .......... .......... .......... .......... ..........  [300]
340 - NOREL    .......... .......... .......... .......... ..........  [300]
913 - NOREL    .......... .......... .......... .......... ..........  [300]
442 - NOREL    .......... .......... .......... .......... ..........  [300]
738 - NOREL    .......... .......... .......... .......... ..........  [300]
321 - NOREL    .......... .......... .......... .......... ..........  [300]
827 - NOREL    .......... .......... .......... .......... ..........  [300]
243A - REL     .......... .......... .......... .......... ..........  [300]
361A - REL     .......... .......... .......... .......... ..........  [300]
126A - REL     .......... .......... .......... .......... ..........  [300]
294A - REL     .......... .......... .......... .......... ..........  [300]
294B - REL     .......... .......... .......... .......... ..........  [300]
297A - REL     .......... .......... .......... .......... ..........  [300]
126B - REL     .......... .......... .......... .......... ..........  [300]
246A - REL     .......... .......... .......... .......... ..........  [300]
246B - REL     .......... .......... .......... .......... ..........  [300]
103B - REL     .......... .......... .......... .......... ..........  [300]
95B - REL      .......... .......... .......... .......... ..........  [300]
103A - REL     .......... .......... .......... .......... ..........  [300]
370A - REL     .......... .......... .......... .......... ..........  [300]
216B - REL     .......... .......... .......... .......... ..........  [300]
216A - REL     .......... .......... .......... .......... ..........  [300]
361B - REL     .......... .......... .......... .......... ..........  [300]
370B - REL     .......... .......... .......... .......... ..........  [300]
95A - REL      .......... .......... .......... .......... ..........  [300]
```

Alignment 1 continuing

```
ELSAL          CACTGATATG TATAACGAGT TATTAAAATT ACGATTCAGC TTGCTGTTTC  [350]
BELPAT         .......... .......... .......... .......... ..........  [350]
SALPAT         .......... .......... .......... .......... ..........  [350]
PVTHAI         .......... .......... .......... .......... ..........  [350]
SALI           .......... .......... .......... .......... ..........  [350]
CYNOMOLGY      .......... .......... ..C....... ...T...T.. ....A.A...  [350]
COLOMBIA       .......... .......... .......... .......... ..........  [350]
I - ADM        .......... .......... .......... .......... ..........  [350]
E - ADM        .......... .......... .......... .......... ..........  [350]
H - ADM        .......... .......... .......... .......... ..........  [350]
D - ADM        .......... .......... .......... .......... ..........  [350]
F - ADM        .......... .......... .......... .......... ..........  [350]
G - ADM        .......... .......... .......... .......... ..........  [350]
J - ADM        .......... .......... .......... .......... ..........  [350]
KA - ADM       .......... .......... .......... .......... ..........  [350]
KB - ADM       .......... .......... .......... .......... ..........  [350]
A - ADM        .......... .......... .......... .......... ..........  [350]
C - ADM        T......... .......... .......... .....A.... ..........  [350]
B - ADM        .......... .......... .......... .......... ..........  [350]
136 - NOREL    .......... .......... .......... .......... ..........  [350]
131 - NOREL    .......... .......... .......... .......... ..........  [350]
212 - NOREL    .......... .......... .......... .......... ..........  [350]
66 - NOREL     .......... .......... .......... .......... ..........  [350]
185 - NOREL    .......... .......... .......... .......... ..........  [350]
340 - NOREL    .......... .......... .......... .......... ..........  [350]
913 - NOREL    .......... .......... .......... .......... ..........  [350]
442 - NOREL    .......... .......... .......... .......... ..........  [350]
738 - NOREL    .......... .......... .......... .......... ..........  [350]
321 - NOREL    .......... .......... .......... .......... ..........  [350]
827 - NOREL    .......... .......... .......... .......... ..........  [350]
243A - REL     .......... .......... .......... .....A.... ..........  [350]
361A - REL     .......... .......... .......... .......... ..........  [350]
126A - REL     .......... .......... .......... .......... ..........  [350]
294A - REL     .......... .......... .......... .......... ..........  [350]
294B - REL     .......... .......... .......... .......... ..........  [350]
297A - REL     .......... .......... .......... .......... ..........  [350]
126B - REL     .......... .......... .......... .......... ..........  [350]
246A - REL     .......... .......... .......... .......... ..........  [350]
246B - REL     .......... .......... .......... .......... ..........  [350]
103B - REL     .......... .......... .......... .......... ..........  [350]
95B - REL      .......... .......... .......... .......... ..........  [350]
103A - REL     .......... .......... .......... .......... ..........  [350]
370A - REL     .......... .......... .......... .......... ..........  [350]
216B - REL     .......... .......... .......... .......... ..........  [350]
216A - REL     .......... .......... .......... .......... ..........  [350]
361B - REL     .......... .......... .......... .......... ..........  [350]
370B - REL     .......... .......... .......... .......... ..........  [350]
95A - REL      .......... .......... .......... .......... ..........  [350]
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Alignment 1 continuing

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ELSAL          GTATTTTTCC TCCACTGAAA AGTGTAGGTA ATCTTTATCA GTACATAT [398]
BELPAT         .......... .......... .......... .......... ........ [398]
SALPAT         .......... .......... .......... .......... ........ [398]
PVTHAI         .......... .......... .......... .......... ........ [398]
SALI           .......... .......... .......... .......... ........ [398]
CYNOMOLGY      ...C...... .......... .......... .......... A....... [398]
COLOMBIA       .......... .......... .......... .......... ........ [398]
I - ADM        .......... .......... .......... .......... ........ [398]
E - ADM        .......... .......... .......... .......... ........ [398]
H - ADM        .......... .......... .......... .......... ........ [398]
D - ADM        .C........ .......... .......... .......... ........ [398]
F - ADM        .C........ .......... .......... .......... ........ [398]
G - ADM        .C........ .......... .......... .......... ........ [398]
J - ADM        .......... .......... .......... .......... ........ [398]
KA - ADM       .......... .......... .......... .......... ........ [398]
KB - ADM       .......... .......... .......... .......... ........ [398]
A - ADM        .......... .......... .......... .......... ........ [398]
C - ADM        .......... .......... .......... .......... ........ [398]
B - ADM        .......... .......... .......... .......... ........ [398]
136 - NOREL    .......... .......... .......... .......... ........ [398]
131 - NOREL    .......... .......... .......... .......... ........ [398]
212 - NOREL    .......... .......... .......... .......... ........ [398]
66 - NOREL     .......... .......... .......... .......... ........ [398]
185 - NOREL    .......... .......... .......... .......... ........ [398]
340 - NOREL    .......... .......... .......... .......... ........ [398]
913 - NOREL    .......... .......... .......... .......... ........ [398]
442 - NOREL    .......... .......... .......... .......... ........ [398]
738 - NOREL    .......... .......... .......... .......... ........ [398]
321 - NOREL    .......... .......... .......... .......... ........ [398]
827 - NOREL    .......... .......... .......... .......... ........ [398]
243A - REL     .......... .......... .......... .......... ........ [398]
361A - REL     .......... .......... .......... .......... ........ [398]
126A - REL     .......... .......... .......... .......... ........ [398]
294A - REL     .......... .......... .......... .......... ........ [398]
294B - REL     .......... .......... .......... .......... ........ [398]
297A - REL     .......... .......... .......... .......... ........ [398]
126B - REL     .......... .......... .......... .......... ........ [398]
246A - REL     .......... .......... .......... .......... ........ [398]
246B - REL     .......... .......... .......... .......... ........ [398]
103B - REL     .......... .......... .......... .......... ........ [398]
95B - REL      .......... .......... .......... .......... ........ [398]
103A - REL     .......... .......... .......... .......... ........ [398]
370A - REL     .......... .......... .......... .......... ........ [398]
216B - REL     .......... .......... .......... .......... ........ [398]
216A - REL     .......... .......... .......... .......... ........ [398]
361B - REL     .......... .......... .......... .......... ........ [398]
370B - REL     .......... .......... .......... .......... ........ [398]
95A - REL      .......... .......... .......... .......... ........ [398]
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Alignment 2: CSP gene, amino acid sequences from Manaus and references Salvador I (SALPAT) and Belém (BELPAT). Belém was the reference strain, identical amino acids are represented by dots, and gaps are represented by dashes. Amino acids unique to strains from Manaus in red, amino acids identical to Salvador I highlighted in yellow.

```
BELPAT     KDGKKAEPKN PRENKLKQP- GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GD [ 58]
B-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
J-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
H-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
A-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
F-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
C-ADM      .......... .........A ......... ......... ......... ......... .. [ 58]
E-ADM      .......... .........A ......... ......... ......... ......... .. [ 58]
G-ADM      .......... .........- ......... ......... ......... ......... .. [ 58]
D-ADM      .......... .........- ......... ......... ......... ....D.... .. [ 58]
I-ADM      .......... .........- ......... ......... ......... ....D.... .. [ 58]
340-NOREL  .......... .........- ......... ......... ......... ......... .. [ 58]
321-NOREL  .......... .........- ......... ......... ......... ......... .. [ 58]
442-NOREL  .......... .........- ......... ......... ......... ......... .. [ 58]
185-NOREL  .......... .........- ......... ......... ......... ......... .. [ 58]
131-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
136-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
212-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
913-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
738-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
827-NOREL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
66-NOREL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
103A-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
243A-REL   .......... .........- ......... ......... ......... ......... .. [ 58]
294B-REL   .......... .........- ......... ......... ......... ......... .. [ 58]
126A-REL   .......... .........A ......... ......... ......... ....D.... .. [ 58]
126B1-REL  .......... .........- ......... ......... ......... ......... .. [ 58]
126B2-REL  .......... .........- ......... ......... ......... ....D.... .. [ 58]
216A-REL   .......... .........- ......... ......... ......... ......... .. [ 58]
216B-REL   .......... .........- ......... ......... ......... ......... .. [ 58]
KA-ADM     .......... .........- ......... ......... ......... ....D.... .. [ 58]
KB-ADM     .......... .........- ......... ......... ......... ....D.... .. [ 58]
297A-REL   .......... .........- ......... ......... ......... ......... .. [ 58]
297B-REL   .......... .........A ......... ......... ......... ......... .. [ 58]
95A-REL    .......... .........- ......... ......... ......... ....D.... .. [ 58]
95B-REL    .......... .........- ......... ......... ......... ......... .. [ 58]
246A-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
246B-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
370A-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
370B-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
361A-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
361B-REL   .......... .........- ......... ......... ......... ....D.... .. [ 58]
SALPAT     .......... .........- ......... ......... ......... ....D.... .. [ 58]
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Alignment 2 continuing

```
BELPAT     RADGQPA GDRADGQPA GDRADGQPA GDRADGQPA GDRAAGQPA GDRAAGQPA GDRADG [116]
B-ADM      ....... ......... ......... ......... ......... ......... ...... [116]
J-ADM      ....... ......... ......... ......... ......... ......... ...... [116]
H-ADM      ....... ......... ......... ......... ......... ......... ...... [116]
A-ADM      ....... ......... ......... ......... ......... ......... ...... [116]
F-ADM      ....... ......... ......... ......... ......... ......... ...... [116]
C-ADM      ....... ....A.... ......... ....A.... ....D.... ......... ...... [116]
E-ADM      ....... ....A.... ......... ....A.... ....D.... ......... ...... [116]
G-ADM      ....... ......... ......... ....A.... ......... ....D.... ....A. [116]
D-ADM      ........ .N....... ......... ......... ....D.... ....D.... ...... [116]
I-ADM      ........ .N....... ......... ......... ....D.... ....D.... ...... [116]
340-NOREL  ....... ......... ......... ......... ......... ......... ...... [116]
321-NOREL  ....... ......... ......... ......... ......... ......... ...... [116]
442-NOREL  ....... ......... ......... ......... ......... ......... ...... [116]
185-NOREL  ....... ......... ......... ......... ......... ......... ...... [116]
131-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
136-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
212-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
913-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
738-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
827-NOREL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
66-NOREL   ..A.... ......... ......... ......... ....D.... ......... ....A. [116]
103A-REL   ....... ......... ......... ......... ....D.... ....D.... ...... [116]
243A-REL   ....... ......... ......... ......... ......... ......... ...... [116]
294B-REL   ....... ......... ......... ......... ......... ......... ...... [116]
126A-REL   ....... ......... ......... ......... ....D.... ....D.... ...... [116]
126B1-REL  ....... ......... ......... ......... ......... ......... ...... [116]
126B2-REL  ....... ......... ......... ......... ....D.... ....D.... ...... [116]
216A-REL   ....... ......... ......... ......... ......... ......... ...... [116]
216B-REL   ....... ......... ......... ......... ......... ......... ...... [116]
KA-ADM     ..A.... ......... ......... ......... ......... ......... ...... [116]
KB-ADM     ..A.... ......... ......... ......... ......... ......... ...... [116]
297A-REL   ....... ......... ......... ......... ......... ......... ...... [116]
297B-REL   ....... ....A.... ......... ....A.... ....D.... ......... ...... [116]
95A-REL    ........ .N....... ......... ......... ....D.... ....D.... ...... [116]
95B-REL    ....... ......... ......... ......... ......... ......... ...... [116]
246A-REL   ....... ......... ......... ......... ....D.... ....D.... ...... [116]
246B-REL   ....... ......... ......... ......... ....D.... ....D.... ...... [116]
370A-REL   ....... ......... ......... ......... ....D.... ....D.... ...... [116]
370B-REL   ..A.... ......... ......... ......... ....D.... ......... ....A. [116]
361A-REL   ..A.... ......... ......... ......... ....D.... ......... ...... [116]
361B-REL   ..A.... ......... ......... ......... ....D.... ......... ....A. [116]
SALPAT     ..A.... ......... ......... ......... ....D.... ......... ....A. [116]
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Alignment 2 continuing

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BELPAT   QPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRADGQPA GDRAAGQPA GDRAAGQPA G [174]
B-ADM    ... ......... ......... ......... ......... ......... ......... . [174]
J-ADM    ... ......... ......... ......... ......... ......... ......... . [174]
H-ADM    ... ......... ......... ......... ......... ......... ......... . [174]
A-ADM    ... ......... ......... ......... ......... ......... ......... . [174]
F-ADM    ... ......... ......... ......... ......... ......... ......... . [174]
C-ADM    ... ......... ....A.... ....D.... ....A.... ....D.... ......... . [174]
E-ADM    ... ......... ....A.... ....D.... ....A.... ....D.... ......... . [174]
G-ADM    ... ....D.... ....A.... ....D.... ....A.... ......... ......... . [174]
D-ADM    ... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
I-ADM    ... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
340-NOREL... ......... ......... ......... ......... ......... ......... . [174]
321-NOREL... ......... ......... ......... ......... ......... ......... . [174]
442-NOREL... ......... ......... ......... ......... ......... ......... . [174]
185-NOREL... ......... ......... ......... ......... ......... ......... . [174]
131-NOREL... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
136-NOREL... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
212-NOREL... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
913-NOREL... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
738-NOREL... ....D.... ......... ....D.... ......... ......... ......... . [174]
827-NOREL... ....D.... ......... ....D.... ......... ......... ......... . [174]
66-NOREL ... ....D.... ....A.... ....D.... ....A.... ....D.... ......... . [174]
103A-REL ... ....D.... ......... ....D.... ......... ......... ......... . [174]
243A-REL ... ......... ......... ......... ......... ......... ......... . [174]
294B-REL ... ......... ......... ......... ......... ......... ......... . [174]
126A-REL ... ....D.... ......... ....D.... ......... ....D.... ....D.... . [174]
126B1-REL... ......... ......... ......... ......... ......... ......... . [174]
126B2-REL... ....D.... ......... ....D.... ......... ....D.... ....D.... . [174]
216A-REL ... ......... ......... ......... ......... ......... ......... . [174]
216B-REL ... ......... ......... ......... ......... ......... ......... . [174]
KA-ADM   ... ......... ......... ......... ......... .......A. ......... . [174]
KB-ADM   ... ......... ......... ......... ......... .......A. ......... . [174]
297A-REL ... ......... ......... ......... ......... ......... ......... . [174]
297B-REL ... ......... ....A.... ....D.... ....A.... ....D.... ......... . [174]
95A-REL  ... ....D.... ......... ....D.... ......... ....D.... ....D.... . [174]
95B-REL  ... ....D.... ....A.... ....D.... ....A.... ......... ......... . [174]
246A-REL ... ....D.... ......... ....D.... ......... ....D.... ......... . [174]
246B-REL ... ....D.... ......... ....D.... ......... ......... ......... . [174]
370A-REL ... ....D.... ......... ....D.... ......... ....D.... ....D.... - [174]
370B-REL ... ....D.... ....A.... ....D.... ....A.... ....D.... ......... . [174]
361A-REL ... ....D.... ....A.... ....D.... ......... ....D.... ......... . [174]
361B-REL ... ....D.... ....A.... ....D.... ....A.... ....D.... ......... . [174]
SALPAT   ... ....D.... ....A.... ....D.... ....A.... ....D.... ......... . [174]
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Alignment 2 continuing

```
BELPAT     DRAAGQPA GDRAAGQPA GNGAGGQAA GGNAGGQGQ NNEGA NAPNEKSVKE YLDKVRAT [232]
B-ADM      ........ ......... ......... ......... ..... .......... ........ [232]
J-ADM      ........ ......... ......... ......... ..... .......... ........ [232]
H-ADM      ........ ......... ......... ......... ..... .......... ........ [232]
A-ADM      ........ ......... ......... ......... ..... .......... ........ [232]
F-ADM      ........ ......... ......... ......... ..... .......... ........ [232]
C-ADM      ........ --------- ......... ......... ..... .......... ........ [232]
E-ADM      ........ --------- ......... ......... ..... .......... ........ [232]
G-ADM      ........ --------- ......... ......... ..... .......... ........ [232]
D-ADM      -------- --------- ......... ......... ..... .......... ........ [232]
I-ADM      -------- --------- ......... ......... ..... .......... ........ [232]
340-NOREL  ........ ......... ......... ......... ..... .......... ........ [232]
321-NOREL  ........ ......... ......... ......... ..... .......... ........ [232]
442-NOREL  ........ ......... ......... ......... ..... .......... ........ [232]
185-NOREL  ........ ......... ......... ......... ..... .......... ........ [232]
131-NOREL  -------- --------- ......... ......... ..... .......... ........ [232]
136-NOREL  -------- --------- ......... ......... ..... .......... ........ [232]
212-NOREL  -------- --------- ......... ......... ..... .......... ........ [232]
913-NOREL  -------- --------- ......... ......... ..... .......... ........ [232]
738-NOREL  ........ --------- ......... ......... ..... .......... ........ [232]
827-NOREL  ........ --------- ......... ......... ..... .......... ........ [232]
66-NOREL   ........ ......... .DR.A..P. ......... ..... .......... ........ [232]
103A-REL   ........ --------- ......... ......... ..... .......... ........ [232]
243A-REL   ........ ......... ......... ......... ..... .......... ........ [232]
294B-REL   ........ ......... ......... ......... ..... .......... ........ [232]
126A-REL   ...D.... --------- ......... ......... ..... .......... ........ [232]
126B1-REL  ........ ......... ......... ......... ..... .......... ........ [232]
126B2-REL  ......A. --------- --------- ......... ..... .......... ........ [232]
216A-REL   ........ ......... ......... ......... ..... .......... ........ [232]
216B-REL   ........ ......... ......... ......... ..... .......... ........ [232]
KA-ADM     ......A. .......A. .DR.A.... ......... ..... .......... ........ [232]
KB-ADM     ......A. .......A. .DR.A.... ......... ..... .......... ........ [232]
297A-REL   ........ ......... ......... ......... ..... .......... ........ [232]
297B-REL   ........ --------- ......... ......... ..... .......... ........ [232]
95A-REL    ......A. --------- --------- ......... ..... .......... ........ [232]
95B-REL    ........ --------- ......... ......... ..... .......... ........ [232]
246A-REL   ........ ......... ......... ......... ..... .......... ........ [232]
246B-REL   ........ --------- ......... ......... ..... .......... ........ [232]
370A-REL   -------- --------- ......... ......... ..... .......... ........ [232]
370B-REL   ........ ......... .DR.A.... ......... ..... .......... ........ [232]
361A-REL   ........ ......... .DR.A.... ......... ..... .......... ........ [232]
361B-REL   ........ ......... .DR.A.... ......... ..... .......... ........ [232]
SALPAT     ........ .......A. .DR.A.... ......... ..... .......... ........ [232]
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Alignment 2 continuing

```
BELPAT    VGTEWTPCSV TCGVGVRVRR RVNAANKKPE DLTLNDLETD VCTMDKCAGI  [282]
B-ADM     .......... .......... .......... .......... ..........  [282]
J-ADM     .......... .......... .......... .......... ..........  [282]
H-ADM     .......... .......... .......... .......... ..........  [282]
A-ADM     .......... .......... .......... .......... ..........  [282]
F-ADM     .......... .......... .......... .......... ..........  [282]
C-ADM     .......... .......... .......... .......... ..........  [282]
E-ADM     .......... .......... .......... .......... ..........  [282]
G-ADM     .......... .......... .......... .......... ..........  [282]
D-ADM     .......... .......... .......... .......... ..........  [282]
I-ADM     .......... .......... .......... .......... ..........  [282]
340-NOREL .......... .......... .......... .......... ..........  [282]
321-NOREL .......... .......... .......... .......... ..........  [282]
442-NOREL .......... .......... .......... .......... ..........  [282]
185-NOREL .......... .......... .......... .......... ..........  [282]
131-NOREL .......... .......... .......... .......... ..........  [282]
136-NOREL .......... .......... .......... .......... ..........  [282]
212-NOREL .......... .......... .......... .......... ..........  [282]
913-NOREL .......... .......... .......... .......... ..........  [282]
738-NOREL .......... .......... .......... .......... ..........  [282]
827-NOREL .......... .......... .......... .......... ..........  [282]
66-NOREL  .......... .......... .......... .......... ..........  [282]
103A-REL  .......... .......... .......... .......... ..........  [282]
243A-REL  .......... .......... .......... .......... ..........  [282]
294B-REL  .......... .......... .......... .......... ..........  [282]
126A-REL  .......... .......... .......... .......... ..........  [282]
126B1-REL .......... .......... .......... .......... ..........  [282]
126B2-REL .......... .......... .......... .......... ..........  [282]
216A-REL  .......... .......... .......... .......... ..........  [282]
216B-REL  .......... .......... .......... .......... ..........  [282]
KA-ADM    .......... .......... .......... .......... ..........  [282]
KB-ADM    .......... .......... .......... .......... ..........  [282]
297A-REL  .......... .......... .......... .......... ..........  [282]
297B-REL  .......... .......... .......... .......... ..........  [282]
95A-REL   .......... .......... .......... .......... ..........  [282]
95B-REL   .......... .......... .......... .......... ..........  [282]
246A-REL  .......... .......... .......... .......... ..........  [282]
246B-REL  .......... .......... .......... .......... ..........  [282]
370A-REL  .......... .......... .......... .......... ..........  [282]
370B-REL  .......... .......... .......... .......... ..........  [282]
361A-REL  .......... .......... .......... .......... ..........  [282]
361B-REL  .......... .......... .......... .......... ..........  [282]
SALPAT    .......... .......... .......... .......... ..........  [282]
```

Alignment 3: CSP gene, nucleotide sequences from Manaus and references Salvador (SALPAT) and Belém (BELPAT). Belém was the reference strain, identical nucleotides are represented by dots, and gaps are represented by dashes. Silent and non-silent mutations are presented in lower and upper cases respectively. Translated amino acid sequence on top of the alignment.

```
             K   D   G   K   K   A   E   P   K   N   P   R   E   N   K   L   K   Q   P

BELPAT     AAG GAT GGA AAG AAA GCA GAA CCA AAA AAT CCA CGT GAA AAT AAG CTG AAA CAA CCA  [ 57]
A-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
B-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
C-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
D-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
E-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ...  [ 57]
F-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
G-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
H-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
I-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
J-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KA-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KB-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
131-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
136-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
185-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
212-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
321-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
340-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
442-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
66-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
738-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
827-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
913-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
103A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B1-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B2-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
243A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
294B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
297A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
297B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SALPAT     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
```

Alignment 3 continuing

```
            -   G   D   R   A   D   G   Q   P   A   G   D   R   A   D   G   Q   P   A
            A

BELPAT      --- GGA GAC AGA GCA GAT GGA CAG CCA GCA GGA GAC AGA GCA GAT GGA CAG CCA GCA  [114]
A-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t ...  [114]
B-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
C-ADM       GCA ..t ..t ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ...  [114]
D-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
E-ADM       GCA ..t ..t ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ...  [114]
F-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
G-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
H-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
I-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
J-ADM       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
KA-ADM      --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
KB-ADM      --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
131-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
136-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
185-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
212-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
321-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
340-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
442-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
66-NOREL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
738-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
827-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
913-NOREL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
103A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
126A-REL    GCA ..t ..t ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ...  [114]
126B1-REL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
126B2-REL   --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
216A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
216B-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
243A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
246A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
246B-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
294B-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
297A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
297B-REL    GCA ..t ..t ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ...  [114]
361A-REL    --- ..t ... ... ... ... ... ... ... ..t ... ..t ... ..t ... ... ... ... ...  [114]
361B-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
370A-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
370B-REL    --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
95A-REL     --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
95B-REL     --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
SALPAT      --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
```

Alignment 3 continuing

```
            G   D   R   A   D   G   Q   P   A   G   D   R   A   A   G   Q   P   A   G
                                                                D
BELPAT      GGT GAT AGA GCA GAT GGA CAA CCA GCA GGA GAT AGA GCA GCT GGA CAG CCA GCA GGA  [171]
A-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
B-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
C-ADM       ... ... ... ... ... ... ..g ... ... ..t ... ... ... ... ... ..a ... ... ..t  [171]
D-ADM       ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
E-ADM       ... ... ... ... ... ... ..g ... ... ..t ... ... ... ... ... ..a ... ... ..t  [171]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
G-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
I-ADM       ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
KA-ADM      ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
KB-ADM      ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
131-NOREL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
136-NOREL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
212-NOREL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
66-NOREL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...  [171]
827-NOREL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
913-NOREL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
103A-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
126A-REL    ... ... ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ... ... ... ..t  [171]
126B1-REL   ... ... ... ... ... ... ..g ... ... ... ... ... ... ... ... ... ... ... ...  [171]
126B2-REL   ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ... ... ... ...  [171]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
216B-REL    ... ... ... ... ... ... ..g ... ... ... ... ... ... ... ... ... ... ... ...  [171]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
246A-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
246B-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
297A-REL    ... ... ... ... ... ... ..g ... ... ... ... ... ... ... ... ... ... ... ...  [171]
297B-REL    ... ... ... ... ... ... ..g ... ... ..t ... ... ... ... ... ..a ... ... ..t  [171]
361A-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ... ... ... ...  [171]
361B-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
370A-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
370B-REL    ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
95A-REL     ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
SALPAT      ..a ..c ... ... ... ... ..g ... ... ..t ... ... ... .A. ... ..a ... ... ...  [171]
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Alignment 3 continuing

```
            D   R   A   D   G   Q   P   A   G   D   R   A   D   G   Q   P   A   G   D
                        A                       N           A

BELPAT      GAT AGA GCA GAT GGA CAG CCA GCA GGA GAC AGA GCA GAT GGA CAG CCA GCA GGA GAC  [228]
A-ADM       ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
B-ADM       ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ..t  [228]
C-ADM       ... ... ... ... ... ... ... ... ..c ..t ... ... .C. ... ... ... ... ..c ..t  [228]
D-ADM       ... ... ... ... ... ... ... ... ..t A.T ... ... ... ... ... ... ... ... ...  [228]
E-ADM       ... ... ... ... ... ... ... ... ..c ..t ... ... .C. ... ... ... ... ..c ..t  [228]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
G-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
I-ADM       ... ... ... ... ... ... ... ... ..t A.T ... ... ... ... ... ... ... ... ...  [228]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
KA-ADM      ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
KB-ADM      ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
131-NOREL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
136-NOREL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
212-NOREL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
66-NOREL    ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
738-NOREL   ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
827-NOREL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
913-NOREL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
103A-REL    ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
126A-REL    ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ..t ..t  [228]
126B1-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
126B2-REL   ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
246A-REL    ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
246B-REL    ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
297B-REL    ... ... ... ... ... ... ... ... ..c ..t ... ... .C. ... ... ... ... ..c ..t  [228]
361A-REL    ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
361B-REL    ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
370A-REL    ... ... ... ... ... ... ... ... ..t ..t ... ... ... ... ... ... ... ... ...  [228]
370B-REL    ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
95A-REL     ... ... ... ... ... ... ... ... ..t A.T ... ... ... ... ... ... ... ... ...  [228]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
SALPAT      ... ... ... .C. ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ...  [228]
```

Alignment 3 continuing

	R	A	D	G	Q	P	A	G	D	R	A	D A	G	Q	P	A	G	D	R	
BELPAT	AGA	GCA	GAT	GGA	CAG	CCA	GCA	GGA	GAC	AGA	GCA	GAT	GGA	CAG	CCA	GCA	GGT	GAT	AGA	[285]
A-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..t	...	...	...	...	[285]
B-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
C-ADM	...	...	...	...	...	...	...	...	..t	...	...	.C.	...	...	...	...	..c	...	...	[285]
D-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
E-ADM	...	...	...	...	...	...	...	...	..t	...	...	.C.	...	...	...	...	..c	...	...	[285]
F-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
G-ADM	...	...	...	...	...	...	...	..t	..t	...	...	.C.	...	..a	...	...	...	...	...	[285]
H-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
I-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
J-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
KA-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
KB-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
131-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
136-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
185-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
212-NOREL	...	...	...	..t	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
321-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
340-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
442-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
66-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	..c	...	[285]
738-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
827-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
913-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
103A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
126A-REL	...	...	...	...	...	...	...	...	..t	...	...	...	...	...	...	...	..a	...	...	[285]
126B1-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
126B2-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
216A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
216B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
243A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
246A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
246B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
294B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
297A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
297B-REL	...	...	...	...	...	...	...	...	..t	...	...	.C.	...	...	...	...	..c	...	...	[285]
361A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	..c	...	[285]
361B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	..c	...	[285]
370A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
370B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	..c	...	[285]
95A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
95B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[285]
SALPAT	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	..c	...	[285]

Alignment 3 continuing

	A	A D	G	Q	P	A	G	D	R	A	A D	G	Q	P	A	G	D	R	A	
BELPAT	GCA	GCT	GGA	CAA	CCA	GCA	GGT	GAT	AGA	GCA	GCT	GGA	CAG	CCA	GCA	GGC	GAT	AGA	GCA	[342]
A-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..t	...	...	...	[342]
B-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..a	...	...	...	[342]
C-ADM	...	.A.	...	..g	...	...	..a	...	...	...	...	...	...	...	...	...	...	...	...	[342]
D-ADM	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
E-ADM	...	.A.	...	..g	...	...	..a	...	...	...	...	...	...	...	...	...	...	...	...	[342]
F-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
G-ADM	...	...	...	..g	...	...	..c	...	...	...	.A.	...	...	...	...	..a	...	...	...	[342]
H-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
I-ADM	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
J-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
KA-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
KB-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
131-NOREL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
136-NOREL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
185-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
212-NOREL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
321-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
340-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
442-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
66-NOREL	...	.A.	...	..g	...	...	...	...	...	...	...	...	..a	...	...	..t	...	...	...	[342]
738-NOREL	...	.A.	...	...	...	...	...	...	...	...	.A.	...	...	...	...	..t	...	...	...	[342]
827-NOREL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..t	...	...	...	[342]
913-NOREL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
103A-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..t	...	...	...	[342]
126A-REL	...	.A.	...	..g	...	...	..a	...	...	...	.A.	...	...	...	...	..a	...	...	...	[342]
126B1-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
126B2-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
216A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
216B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
243A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
246A-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..t	...	...	...	[342]
246B-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..t	...	...	...	[342]
294B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
297A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
297B-REL	...	.A.	...	..g	...	...	..a	...	...	...	...	...	...	...	...	...	...	...	...	[342]
361A-REL	...	.A.	...	..g	...	...	...	...	...	...	...	...	..A	...	...	..t	...	...	...	[342]
361B-REL	...	.A.	...	..g	...	...	...	...	...	...	...	...	..A	...	...	..t	...	...	...	[342]
370A-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
370B-REL	...	.A.	...	..g	...	...	...	...	...	...	...	...	..A	...	...	..t	...	...	...	[342]
95A-REL	...	.A.	...	...	...	...	..a	...	...	...	.A.	...	...	...	...	..a	..c	...	...	[342]
95B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[342]
SALPAT	...	.A.	...	..g	...	...	...	...	...	...	...	...	..A	...	...	..t	...	...	...	[342]

Alignment 3 continuing

```
           D   G   Q   P   A   G   D   R   A   A   G   Q   P   A   G   D   R   A   D
           A                                   D                                   A

BELPAT     GAT GGA CAG CCA GCA GGA GAT AGA GCA GCT GGA CAG CCA GCA GGC GAT AGA GCA GAT  [399]
A-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ... ...  [399]
B-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ... ...  [399]
C-ADM      ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ..t ... ... ... .C.  [399]
D-ADM      ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
E-ADM      ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ..t ... ... ... .C.  [399]
F-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
G-ADM      .C. ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
H-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
I-ADM      ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
J-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
KA-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
KB-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
131-NOREL  ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
136-NOREL  ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
185-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
212-NOREL  ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
321-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
340-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
442-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
66-NOREL   .C. ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
738-NOREL  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ..a ... ... ... ...  [399]
827-NOREL  ... ... ..a ... ... ... ... ... ... .A. ... ... ... ... ..a ..c ... ... ...  [399]
913-NOREL  ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
103A-REL   ... ... ..a ... ... ... ... ... ... .A. ... ... ... ... ..a ..c ... ... ...  [399]
126A-REL   ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
126B1-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
126B2-REL  ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
216A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
216B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
243A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
246A-REL   ... ... ..a ... ... ... ... ... ... .A. ... ... ... ... ..a ... ... ... ...  [399]
246B-REL   ... ... ..a ... ... ... ... ... ... .A. ... ... ... ... ..a ..c ... ... ...  [399]
294B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
297A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
297B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ..a ... ... ..t ... ... .C.  [399]
361A-REL   ... ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
361B-REL   .C. ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
370A-REL   ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
370B-REL   .C. ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
95A-REL    ... ... ... ... ... ... ..c ... ... .A. ... ... ... ... ..t ... ... ... ...  [399]
95B-REL    ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
SALPAT     .C. ... ... ... ... ..c ... ... ... .A. ... ... ... ... ..a ... ... ... .C.  [399]
```

Alignment 3 continuing

```
            G   Q   P   A   G   D   R   A   A   G   Q   P   A   G   D   R   A   D   G
                                            D                                   A

BELPAT      GGA CAG CCA GCA GGA GAT AGA GCA GCT GGA CAA CCA GCA GGA GAT AGA GCA GAT GGA  [456]
A-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
B-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
C-ADM       ... ..a ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... .C. ...  [456]
D-ADM       ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
E-ADM       ... ..a ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... .C. ...  [456]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
G-ADM       ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ... ... ... ... .C. ...  [456]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
I-ADM       ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
KA-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
KB-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
131-NOREL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
136-NOREL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
212-NOREL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
66-NOREL    ... ... ... ... ..c ... ... ... .A. ... ..g ... ... ... ... ... ... .C. ...  [456]
738-NOREL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ... ... ... ... ... ...  [456]
827-NOREL   ... ... ... ... ... ..c ... ... .A. ... ..g ... ... ... ..c ... ... ... ...  [456]
913-NOREL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
103A-REL    ... ... ... ... ... ..c ... ... .A. ... ..g ... ... ... ..c ... ... ... ...  [456]
126A-REL    ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ...  [456]
126B1-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
126B2-REL   ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
246A-REL    ... ... ... ... ... ..c ... ... .A. ... ..g ... ... ... ..c ... ... ... ...  [456]
246B-REL    ... ... ... ... ... ..c ... ... .A. ... ..g ... ... ... ..c ... ... ... ...  [456]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
297B-REL    ... ..a ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... .C. ...  [456]
361A-REL    ... ... ... ... ..c ... ... ... .A. ... ..g ... ... ... ... ... ... ... ...  [456]
361B-REL    ... ... ... ... ..c ... ... ... .A. ... ..g ... ... ... ... ... ... .C. ...  [456]
370A-REL    ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
370B-REL    ... ... ... ... ..c ... ... ... .A. ... ..g ... ... ... ... ... ... .C. ...  [456]
95A-REL     ... ..a ... ... ... ... ... ... .A. ... ..g ... ... ..t ... ... ... ... ...  [456]
95B-REL     ... ..a ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... .C. ...  [456]
SALPAT      ... ... ... ... ..c ... ... ... .A. ... ..g ... ... ... ... ... ... .C. ...  [456]
```

Alignment 3 continuing

	Q	P	A	G	D	R	A	A D	G	Q	P A	A	G	D	R	A	A D	G	Q	
BELPAT	CAA	CCA	GCA	GGA	GAT	AGA	GCA	GCT	GGA	CAG	CCA	GCA	GGA	GAT	AGA	GCA	GCT	GGA	CAG	[513]
A-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
B-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
C-ADM	..g	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
D-ADM	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
E-ADM	..g	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
F-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
G-ADM	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
H-ADM	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
I-ADM	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
J-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
KA-ADM	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	[513]
KB-ADM	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	[513]
131-NOREL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
136-NOREL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
185-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
212-NOREL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
321-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
340-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
442-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
66-NOREL	...	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
738-NOREL	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
827-NOREL	..g	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
913-NOREL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
103A-REL	..g	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
126A-REL	..g	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	.A.	...	...	[513]
126B1-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
126B2-REL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
216A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
216B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
243A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
246A-REL	..g	...	...	...	..c	...	...	.A.	...	...	...	...	...	..c	...	...	...	...	...	[513]
246B-REL	..g	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
294B-REL	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
297A-REL	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
297B-REL	..g	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
361A-REL	...	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
361B-REL	...	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
370A-REL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
370B-REL	...	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]
95A-REL	..g	...	...	...	..c	...	...	.A.	...	..a	...	...	...	...	...	...	.A.	...	...	[513]
95B-REL	..g	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
SALPAT	...	...	...	...	...	...	...	.A.	...	..a	...	...	...	...	...	...	...	...	...	[513]

Alignment 3 continuing

```
            P   A   G   D   R   A   A   G   Q   P   A   G   D   R   A   A   G   Q   P
                                    D           A                                   A

BELPAT      CCA GCA GGA GAT AGA GCA GCT GGA CAG CCA GCA GGA GAT AGA GCA GCT GGA CAG CCA  [570]
A-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
B-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
C-ADM       ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
D-ADM       ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
E-ADM       ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
G-ADM       ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
I-ADM       ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
KA-ADM      ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... G..  [570]
KB-ADM      ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... G..  [570]
131-NOREL   ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
136-NOREL   ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
212-NOREL   ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
913-NOREL   ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
126A-REL    ... ... ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- ---  [570]
126B1-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
126B2-REL   ... ... ... ... ... ... ... ... ... G.. ... --- --- --- --- --- --- --- ---  [570]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
297B-REL    ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
370A-REL    ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
370B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
95A-REL     ... ... ... ... ... ... ... ... ... G.. ... --- --- --- --- --- --- --- ---  [570]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
SALPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G..  [570]
```

Alignment 3 continuing

	A	G	N	G	A	G	G	Q	A	A	G	G	N	A	G	G	Q	G	Q	
			D	R		A			P											
BELPAT	GCA	GGA	AAT	GGT	GCA	GGT	GGA	CAG	GCA	GCA	GGA	GGA	AAC	GCA	GGA	GGA	CAG	GGA	CAA	[627]
A-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
B-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
C-ADM	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
D-ADM	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
E-ADM	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
F-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
G-ADM	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
H-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
I-ADM	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
J-ADM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
KA-ADM	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
KB-ADM	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
131-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
136-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
185-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
212-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
321-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
340-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
442-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
66-NOREL	...	...	G..	A.A	...	.C.	...	...	C..	...	...	...	...	...	...	...	...	...	...	[627]
738-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
827-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
913-NOREL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
103A-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
126A-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
126B1-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
126B2-REL	---	---	---	---	---	---	---	---	---	---	...	...	...	...	...	...	...	...	...	[627]
216A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
216B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
243A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
246A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
246B-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
294B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
297A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
297B-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
361A-REL	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
361B-REL	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
370A-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
370B-REL	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
95A-REL	---	---	---	---	---	---	---	---	---	---	...	...	...	...	...	...	...	...	...	[627]
95B-REL	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]
SALPAT	...	...	G..	A.A	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	[627]

Alignment 3 continuing

```
                N   N   E   G   A   N   A   P   N   E   K   S   V   K   E   Y   L   D   K

BELPAT      AAT AAT GAA GGT GCG AAT GCC CCA AAT GAA AAG TCT GTG AAA GAA TAC CTA GAT AAA  [684]
A-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
B-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
C-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
D-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
E-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
G-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
I-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
KA-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
KB-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
131-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
136-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
126B1-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
126B2-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
297B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
370B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
95A-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
SALPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [684]
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Alignment 3 continuing

```
            V   R   A   T   V   G   T   E   W   T   P   C   S   V   T   C   G   V   G

BELPAT     GTT AGA GCT ACC GTT GGC ACC GAA TGG ACT CCA TGC AGT GTA ACC TGT GGA GTG GGT  [741]
A-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
B-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
C-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
D-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
E-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
F-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
G-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
H-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
I-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
J-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
KA-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
KB-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
131-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
136-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
185-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
212-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
321-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
340-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
442-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
66-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
738-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
827-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
913-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
103A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
126A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
126B1-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
126B2-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
216A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
216B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
243A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
246A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
246B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
294B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
297A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
297B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
361A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
361B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
370A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
370B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
95A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
95B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
SALPAT     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [741]
```

Alignment 3 continuing

```
                V   R   V   R   R   R   V   N   A   A   N   K   K   P   E   D   L   T   L

BELPAT      GTA AGA GTC AGA AGA AGA GTT AAT GCA GCT AAC AAA AAA CCA GAG GAT CTT ACT TTG  [798]
A-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
B-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
C-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
D-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
E-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
F-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
G-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
H-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
I-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
J-ADM       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KA-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KB-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
131-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
136-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126B1-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126B2-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
297B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
370B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
95A-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
SALPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
```

Alignment 3 continuing

```
           N   D   L   E   T   D   V   C   T   M   D   K   C   A   G   I

BELPAT    AAT GAC CTT GAG ACT GAT GTT TGT ACA ATG GAT AAG TGT GCT GGC ATA TT [848]
A-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
B-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
C-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
D-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
E-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
F-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
G-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
H-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
I-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
J-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
KA-ADM    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
KB-ADM    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
131-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
136-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
185-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
212-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
321-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
340-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
442-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
66-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
738-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
827-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
913-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
103A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
126A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
126B1-REL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
126B2-REL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
216A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
216B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
243A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
246A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
246B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
294B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
297A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
297B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
361A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
361B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
370A-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
370B-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
95A-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
95B-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
SALPAT    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [848]
```

Alignment 4: CSP amino acid sequences from Manaus, references Belém (BELPAT) and Salvador I (SALPAT), and sequences retrieved from Gene Bank. Identical amino acids represented by dots and gaps represented by dashes.

```
BELPAT        KDGKKAEPKN PRENKLKQP- GDRADGQPAG DRADGQPAGD RADGQPAGDR AAGQPAGDRA DG [ 62]
NYUTHAI       .......... .........- .......... .......... .......... .-........ .. [ 62]
G24           .......... .........T .......... ...A...... .......... .D........ .. [ 62]
SOL83         .......... .........- V.......-. .......... .......... .......... .. [ 62]
KCSP0067      .G........ .........- .......... ...A...... G.A......G ........G. A. [ 62]
KNIHCSP078099 .G........ .........- .......... ...A...... G.A......G ........G. A. [ 62]
KCSP9775      .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
KCSP966       .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
KCSP967       .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
KCSP9550      .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
KNIHCSP99099  .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
CH5           .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
KCSP9674      .......... .........- .......... ...A...... G.A......G ........G. A. [ 62]
CH4           .......... .........- .......... ...A...... G.A......G .......... A. [ 62]
PH46          .......... .........G .......... .......... ..A....... .D........ .. [ 62]
SOL101        .......... .........A .......... .......... ..A....... .D........ .. [ 62]
NKS           .......... .........- .......... .......... .....A..NG .G........ A. [ 62]
KNIHCSP99161  .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KNIHCSP99056  .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP9621    .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP9625    .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP9711    .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP9642    .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KCSP971       .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP9611    .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP971     .......... .........- .......A.. NG.G...... ..A......G .......... .. [ 62]
KPVCSP98288   .......... .........- .......A.. NG.G...... ..A......G ........G. .. [ 62]
KPVCSP9633    .......... .........- .......A.. NG.G...... .........G .......... .. [ 62]
B             .......... .........- .......... .......... .......... .......... .. [ 62]
J             .......... .........- .......... .......... .......... .......... .. [ 62]
H             .......... .........- .......... .......... .......... .......... .. [ 62]
A             .......... .........- .......... .......... .......... .......... .. [ 62]
F             .......... .........- .......... .......... .......... .......... .. [ 62]
C             .......... .........A .......... .......... .......... .......... .. [ 62]
E             .......... .........A .......... .......... .......... .......... .. [ 62]
G             .......... .........- .......... .......... .......... .......... .. [ 62]
D             .......... .........- .......... .......... .......... .D........ .. [ 62]
I             .......... .........- .......... .......... .......... .D........ .. [ 62]
340           .......... .........- .......... .......... .......... .......... .. [ 62]
321           .......... .........- .......... .......... .......... .......... .. [ 62]
442           .......... .........- .......... .......... .......... .......... .. [ 62]
185           .......... .........- .......... .......... .......... .......... .. [ 62]
131           .......... .........- .......... .......... .......... .D........ .. [ 62]
136           .......... .........- .......... .......... .......... .D........ .. [ 62]
212           .......... .........- .......... .......... .......... .D........ .. [ 62]
913           .......... .........- .......... .......... .......... .D........ .. [ 62]
738           .......... .........- .......... .......... .......... .D........ .. [ 62]
827           .......... .........- .......... .......... .......... .D........ .. [ 62]
66            .......... .........- .......... .......... .......... .D........ A. [ 62]
103A          .......... .........- .......... .......... .......... .D........ .. [ 62]
243A          .......... .........- .......... .......... .......... .......... .. [ 62]
294B          .......... .........- .......... .......... .......... .......... .. [ 62]
126A          .......... .........A .......... .......... .......... .D........ .. [ 62]
126B1         .......... .........- .......... .......... .......... .......... .. [ 62]
126B2         .......... .........- .......... .......... .......... .D........ .. [ 62]
216A          .......... .........- .......... .......... .......... .......... .. [ 62]
216B          .......... .........- .......... .......... .......... .......... .. [ 62]
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KA            .......... .........- .......... .......... .......... .D........ A. [ 62]
KB            .......... .........- .......... .......... .......... .D........ A. [ 62]
297A          .......... .........- .......... .......... .......... .......... .. [ 62]
297B          .......... .........A .......... .......... .......... .......... .. [ 62]
95A           .......... .........- .......... .......... .......... .D........ .. [ 62]
95B           .......... .........- .......... .......... .......... .......... .. [ 62]
246A          .......... .........- .......... .......... .......... .D........ .. [ 62]
246B          .......... .........- .......... .......... .......... .D........ .. [ 62]
370A          .......... .........- .......... .......... .......... .D........ .. [ 62]
370B          .......... .........- .......... .......... .......... .D........ A. [ 62]
361A          .......... .........- .......... .......... .......... .D........ A. [ 62]
361B          .......... .........- .......... .......... .......... .D........ A. [ 62]
SALPAT        .......... .........- .......... .......... .......... .D........ A. [ 62]
YOELIICS      ..P..DD.PK EAQ...N..- --VVADENVD QG-P.A.Q.- --P.A.Q.-P G.P.G----- -- [ 62]
YONIGCS       .EE...D.PK EAQ......A --AVADPN.P VADPNA.VA. --PNA.VA.P N.PVAD---- -- [ 62]
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Alignment 4 continuing

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BELPAT        QPAGDRADGQ PAGDRADGQP AGDRADGQPA GDRAAGQPAG DRAAGQPAGD RADGQPAGDR AA [124]
NYUTHAI       .......... .......... .......... ....-..... ...-...... .......... .- [124]
G24           .......... .......... .......... ....D..... .......... ..A....... P. [124]
SOL83         .......A.. .......... .....A.... ....D..... .......... .......... .. [124]
KCSP0067      .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KNIHCSP078099 .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KCSP9775      .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KCSP966       .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KCSP967       .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KCSP9550      .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
KNIHCSP99099  .......A.. ....G.A... ...G.A.... .......... .......... ..A....... .. [124]
CH5           .......A.. ....G.A... ...G.A.... ..G....... .G........ G.A....... .. [124]
KCSP9674      .......A.. ......A... ...G.A.... .......... .......... ..A....... .. [124]
CH4           .......... ....G.A... ...G.A.... ....D..... .......... ..A....... .D [124]
PH46          .......A.. .......... .....A.... ....D..... .......... ..A....... .D [124]
SOL101        .......A.. .......... .....A.... ....D..... .......... ..A....... .D [124]
NKS           .....G.A.. .......... .....A.... ....D..... .......... .......... .. [124]
KNIHCSP99161  .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KNIHCSP99056  .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9621    .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9625    .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9711    .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9642    .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KCSP971       .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9611    .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP971     .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP98288   .A..NG.G.. ......A... ...G.A.... ....D..... .......... .......... .. [124]
KPVCSP9633    .A..NG.G.. ......A... ...G.A.... ....D..... ...D...... .......... .. [124]
B             .......... .......... .......... .......... .......... .......... .. [124]
J             .......... .......... .......... .......... .......... .......... .. [124]
H             .......... .......... .......... .......... .......... .......... .. [124]
A             .......... .......... .......... .......... .......... .......... .. [124]
F             .......... .......... .......... .......... .......... .......... .. [124]
C             .......A.. .......... .....A.... ....D..... .......... .......... .. [124]
E             .......A.. .......... .....A.... ....D..... .......... .......... .. [124]
G             .......... .......... .....A.... .......... ...D...... ..A....... .D [124]
D             ....N..... .......... .......... ....D..... ...D...... .......... .D [124]
I             ....N..... .......... .......... ....D..... ...D...... .......... .D [124]
340           .......... .......... .......... .......... .......... .......... .. [124]
321           .......... .......... .......... .......... .......... .......... .. [124]
442           .......... .......... .......... .......... .......... .......... .. [124]
185           .......... .......... .......... .......... .......... .......... .. [124]
131           .......... .......... .......... ....D..... ...D...... .......... .D [124]
136           .......... .......... .......... ....D..... ...D...... .......... .D [124]
212           .......... .......... .......... ....D..... ...D...... .......... .D [124]
913           .......... .......... .......... ....D..... ...D...... .......... .D [124]
738           .......... .......... .......... ....D..... ...D...... .......... .D [124]
827           .......... .......... .......... ....D..... ...D...... .......... .D [124]
66            .......... .......... .......... ....D..... .......... ..A....... .D [124]
103A          .......... .......... .......... ....D..... ...D...... .......... .D [124]
243A          .......... .......... .......... .......... .......... .......... .. [124]
294B          .......... .......... .......... .......... .......... .......... .. [124]
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126A          .......... .......... .......... ....D..... ...D...... .......... .D [124]
126B1         .......... .......... .......... .......... .......... .......... .. [124]
126B2         .......... .......... .......... ....D..... ...D...... .......... .D [124]
216A          .......... .......... .......... .......... .......... .......... .. [124]
216B          .......... .......... .......... .......... .......... .......... .. [124]
KA            .......... .......... .......... .......... .......... .......... .. [124]
KB            .......... .......... .......... .......... .......... .......... .. [124]
297A          .......... .......... .......... .......... .......... .......... .. [124]
297B          .......A.. .......... .....A.... ....D..... .......... .......... .. [124]
95A           ....N..... .......... .......... ....D..... ...D...... .......... .D [124]
95B           .......... .......... .......... .......... .......... .......... .D [124]
246A          .......... .......... .......... ....D..... ...D...... .......... .D [124]
246B          .......... .......... .......... ....D..... ...D...... .......... .D [124]
370A          .......... .......... .......... ....D..... ...D...... .......... .D [124]
370B          .......... .......... .......... ....D..... .......... ..A....... .D [124]
361A          .......... .......... .......... ....D..... .......... .......... .D [124]
361B          .......... .......... .......... ....D..... .......... ..A....... .D [124]
SALPAT        .......... .......... .......... ....D..... .......... ..A....... .D [124]
YOELIICS      ---PGAPQ.- .GAPQG--PG .PQG-P.A.Q .-PG.P.G-P GAPQ.-.GAP QG-------- -P [124]
YONIGCS       ---PNAPVAD .NAPV..-PN .PVADPNA.V A.PN.PVADP NAPVAD.NAP V..------- -P [124]
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Alignment 4 continuing

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BELPAT        GQPAGDRADG QPAGDRAAGQ PAGDRADGQP AGDRAAGQPA GDRAAGQPAG DRAAGQPAGD RA [186]
NYUTHAI       .......... .......-.. .......... .....-.... ....-..... ...-...... .. [186]
G24           ........A. .......D.. .......... .....D.... .......... .......... .. [186]
SOL83         ........A. .......D.. ......A... .......... ....D..... .........-- -- [186]
KCSP0067      ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KNIHCSP078099 ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KCSP9775      ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KCSP966       ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KCSP967       ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KCSP9550      ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KNIHCSP99099  ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
CH5           ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
KCSP9674      ........A. .......... .......... .......... .......... NG.G..A.-- -- [186]
CH4           ........A. .......D.. ......A... .......... .......... NG.G..A.-- -- [186]
PH46          ........A. .......D.. ......A... .......... .......... NG.G..A.-- -- [186]
SOL101        ........A. .......D.. ......A... .......... .......... ...D....-- -- [186]
NKS           ..A..NG.G. .A..NG.G.. ......A... .......... .......... .......... .. [186]
KNIHCSP99161  ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KNIHCSP99056  ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP9621    ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP9625    ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP9711    ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP9642    ..A..NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
KCSP971       ..A..NG.G. .A..NG.G.. ......G... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP9611    ..A..NG.G. .A..NG.G.. ......G... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP971     ..A..NG.G. .A..NG.G.. ......G... ........A. .NG.G..A.. NG.G..A..N G. [186]
KPVCSP98288   ..A..NG.G. .A..NG.G.. .....TA... ..N.TT..A. .NG.G..A.. KG.G..A.RN G. [186]
KPVCSP9633    .....NG.G. .A..NG.G.. ......A... ........A. .NG.G..A.. NG.G..A..N G. [186]
B             .......... .......... .......... .......... .......... .......... .. [186]
J             .......... .......... .......... .......... .......... .......... .. [186]
H             .......... .......... .......... .......... .......... .......... .. [186]
A             .......... .......... .......... .......... .......... .......... .. [186]
F             .......... .......... .......... .......... .......... .......... .. [186]
C             ........A. .......D.. ......A... .....D.... .......... .........-- -- [186]
E             ........A. .......D.. ......A... .....D.... .......... .........-- -- [186]
G             ........A. .......D.. ......A... .......... .......... .........-- -- [186]
D             .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
I             .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
340           .......... .......... .......... .......... .......... .......... .. [186]
321           .......... .......... .......... .......... .......... .......... .. [186]
442           .......... .......... .......... .......... .......... .......... .. [186]
185           .......... .......... .......... .......... .......... .......... .. [186]
131           .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
136           .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
212           .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
913           .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
738           .......... .......D.. .......... .......... .......... .........-- -- [186]
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827          .......... .......D.. .......... .......... .......... .........-- -- [186]
66           ........A. .......D.. ......A... .....D.... .......... .......... .. [186]
103A         .......... .......D.. .......... .......... .......... .........-- -- [186]
243A         .......... .......... .......... .......... .......... .......... .. [186]
294B         .......... .......... .......... .......... .......... .......... .. [186]
126A         .......... .......D.. .......... .....D.... ....D..... ...D....-- -- [186]
126B1        .......... .......... .......... .......... .......... .......... .. [186]
126B2        .......... .......D.. .......... .....D.... ....D..... ......A.-- -- [186]
216A         .......... .......... .......... .......... .......... .......... .. [186]
216B         .......... .......... .......... .......... .......... .......... .. [186]
KA           .......... .......... .......... ........A. .......... ......A... .. [186]
KB           .......... .......... .......... ........A. .......... ......A... .. [186]
297A         .......... .......... .......... .......... .......... .......... .. [186]
297B         ........A. .......D.. ......A... .....D.... .......... .........-- -- [186]
95A          .......... .......D.. .......... .....D.... ....D..... ......A.-- -- [186]
95B          ........A. .......D.. ......A... .......... .......... .........-- -- [186]
246A         .......... .......D.. .......... .....D.... .......... .......... .. [186]
246B         .......... .......D.. .......... .......... .......... .........-- -- [186]
370A         .......... .......D.. .......... .....D.... ....D....- ---------- -- [186]
370B         ........A. .......D.. ......A... .....D.... .......... .......... .. [186]
361A         ........A. .......D.. .......... .....D.... .......... .......... .. [186]
361B         ........A. .......D.. ......A... .....D.... .......... .......... .. [186]
SALPAT       ........A. .......D.. ......A... .....D.... .......... .......... .. [186]
YOELIICS     .A.Q.-PGAP .G-PGAPQ.- .GAPQG-PGA PQEPPQQP.Q QP-PQ-..PQ QPPQQP.QQP PQ [186]
YONIGCS      NA.VA.PNAP RADPNAPRAD .NAP...PNA PRADPNAP.Q QPQPQP..KP QPKPQPKPQP KP [186]
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Alignment 4 continuing

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BELPAT       AGQPAGNGAG GQAA------ ---------- ----GGNAGG QGQNNEGANA PNEKSVKEYL DK [248]
NYUTHAI      -......... ..-------- ---------- ----...... .......... .......... .. [248]
G24          .....A.... ...------- ---------- ----...... .......... .......... .. [248]
SOL83        -----..... ....------ ---------- GGNA...... .......... .......... .. [248]
KCSP0067     ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KNIHCSP078099 ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KCSP9775     ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KCSP966      ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KCSP967      ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KCSP9550     ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
KNIHCSP99099 ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
CH5          ---------- ----GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KCSP9674     ---------- ----GGNAAN KKAEDAGGNA GGNA...... .......... .......... .. [248]
CH4          ---------- ----GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
PH46         ---------- ---------- ------GGNA GGNA...... .......... .......... .. [248]
SOL101       ---------- ---------- ---------- GGNA...... .......... .......... .. [248]
NKS          ...A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KNIHCSP99161 G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KNIHCSP99056 G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9621   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9625   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9711   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9642   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KCSP971      G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9611   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP971    G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP98288  G..A...... .P..GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
KPVCSP9633   G..A...... ....GGNAAN KKAEDAGGNA ----...... .......... .......... .. [248]
B            .......... ....------ ---------- ----...... .......... .......... .. [248]
J            .......... ....------ ---------- ----...... .......... .......... .. [248]
H            .......... ....------ ---------- ----...... .......... .......... .. [248]
A            .......... ....------ ---------- ----...... .......... .......... .. [248]
F            .......... ....------ ---------- ----...... .......... .......... .. [248]
C            -----..... ....------ ---------- ----...... .......... .......... .. [248]
E            -----..... ....------ ---------- ----...... .......... .......... .. [248]
G            -----..... ....------ ---------- ----...... .......... .......... .. [248]
D            -----..... ....------ ---------- ----...... .......... .......... .. [248]
I            -----..... ....------ ---------- ----...... .......... .......... .. [248]
340          .......... ....------ ---------- ----...... .......... .......... .. [248]
321          .......... ....------ ---------- ----...... .......... .......... .. [248]
442          .......... ....------ ---------- ----...... .......... .......... .. [248]
185          .......... ....------ ---------- ----...... .......... .......... .. [248]
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131          ----..... ...------ ---------- ----..... .......... .......... .. [248]
136          ----..... ...------ ---------- ----..... .......... .......... .. [248]
212          ----..... ...------ ---------- ----..... .......... .......... .. [248]
913          ----..... ...------ ---------- ----..... .......... .......... .. [248]
738          ----..... ...------ ---------- ----..... .......... .......... .. [248]
827          ----..... ...------ ---------- ----..... .......... .......... .. [248]
66           ......DR.A ..P.------ ---------- ----..... .......... .......... .. [248]
103A         ----..... ...------ ---------- ----..... .......... .......... .. [248]
243A         .......... ...------ ---------- ----..... .......... .......... .. [248]
294B         .......... ...------ ---------- ----..... .......... .......... .. [248]
126A         ----..... ...------ ---------- ----..... .......... .......... .. [248]
126B1        .......... ...------ ---------- ----..... .......... .......... .. [248]
126B2        ---------- ---------- ---------- ----..... .......... .......... .. [248]
216A         .......... ...------ ---------- ----..... .......... .......... .. [248]
216B         .......... ...------ ---------- ----..... .......... .......... .. [248]
KA           ...A..DR.A ...------ ---------- ----..... .......... .......... .. [248]
KB           ...A..DR.A ...------ ---------- ----..... .......... .......... .. [248]
297A         .......... ...------ ---------- ----..... .......... .......... .. [248]
297B         ----..... ...------ ---------- ----..... .......... .......... .. [248]
95A          ---------- ---------- ---------- ----..... .......... .......... .. [248]
95B          ----..... ...------ ---------- ----..... .......... .......... .. [248]
246A         .......... ...------ ---------- ----..... .......... .......... .. [248]
246B         ----..... ...------ ---------- ----..... .......... .......... .. [248]
370A         ----..... ...------ ---------- ----..... .......... .......... .. [248]
370B         ......DR.A ...------ ---------- ----..... .......... .......... .. [248]
361A         ......DR.A ...------ ---------- ----..... .......... .......... .. [248]
361B         ......DR.A ...------ ---------- ----..... .......... .......... .. [248]
SALPAT       ...A..DR.A ...------ ---------- ----..... .......... .......... .. [248]
YOELIICS     QPR.QPD--- ----G-NNNN -------NN- ------.N-- -.N...DSYV .SAEQIL.FV KQ [248]
YONIGCS      QP..QP---- ----GDNNNN NNNNNN-NN- ------.N-- -.N...DSYV .SAEQIL.FV KQ [248]
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Alignment 4 continuing

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BELPAT       VRATVGTEWT PCSVTCGVGV RVRRRVNAAN KKPEDLTLND LETDVCTMDK CAGI [302]
NYUTHAI      .......... .......... .......... .......... .......... .... [302]
G24          .......... .......... .......... .......... .......... .... [302]
SOL83        .......... .......... .......... .......... .......... .... [302]
KCSP0067     .......... .......... .......... .......... .......... .... [302]
KNIHCSP078099 .......... .......... .......... .......... .......... .... [302]
KCSP9775     .......... .......... .......... .......... .......... .... [302]
KCSP966      .......... .......... .......... .......... .......... .... [302]
KCSP967      .......... .......... .......... .......... .......... .... [302]
KCSP9550     .......... .......... .......... .......... .......... .... [302]
KNIHCSP99099 .......... .......... .......... .......... .......... .... [302]
CH5          .......... .......... .......... .......... .......... .... [302]
KCSP9674     .......... .......... .......... .......... .......... .... [302]
CH4          .......... .......... .......... .......... .......... .... [302]
PH46         .......... .......... .......... .......... .......... .... [302]
SOL101       .......... .......... .......... .......... .......... .... [302]
NKS          .......... .......... .......... .......... .......... .... [302]
KNIHCSP99161 .......... .......... .......... .......... .......... .... [302]
KNIHCSP99056 .......... .......... .......... .......... .......... .... [302]
KPVCSP9621   .......... .......... .......... .......... .......... .... [302]
KPVCSP9625   .......... .......... .......... .......... .......... .... [302]
KPVCSP9711   .......... .......... .......... .......... .......... .... [302]
KPVCSP9642   .......... .......... .......... .......... .......... .... [302]
KCSP971      .......... .......... .......... .......... .......... .... [302]
KPVCSP9611   .......... .......... .......... .......... .......... .... [302]
KPVCSP971    .......... .......... .......... .......... .......... .... [302]
KPVCSP98288  .......... .......... .......... .......... .......... .... [302]
KPVCSP9633   .......... .......... .......... .......... .......... .... [302]
B            .......... .......... .......... .......... .......... .... [302]
J            .......... .......... .......... .......... .......... .... [302]
H            .......... .......... .......... .......... .......... .... [302]
A            .......... .......... .......... .......... .......... .... [302]
F            .......... .......... .......... .......... .......... .... [302]
C            .......... .......... .......... .......... .......... .... [302]
E            .......... .......... .......... .......... .......... .... [302]
G            .......... .......... .......... .......... .......... .... [302]
D            .......... .......... .......... .......... .......... .... [302]
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I            .......... .......... .......... .......... .......... .... [302]
340          .......... .......... .......... .......... .......... .... [302]
321          .......... .......... .......... .......... .......... .... [302]
442          .......... .......... .......... .......... .......... .... [302]
185          .......... .......... .......... .......... .......... .... [302]
131          .......... .......... .......... .......... .......... .... [302]
136          .......... .......... .......... .......... .......... .... [302]
212          .......... .......... .......... .......... .......... .... [302]
913          .......... .......... .......... .......... .......... .... [302]
738          .......... .......... .......... .......... .......... .... [302]
827          .......... .......... .......... .......... .......... .... [302]
66           .......... .......... .......... .......... .......... .... [302]
103A         .......... .......... .......... .......... .......... .... [302]
243A         .......... .......... .......... .......... .......... .... [302]
294B         .......... .......... .......... .......... .......... .... [302]
126A         .......... .......... .......... .......... .......... .... [302]
126B1        .......... .......... .......... .......... .......... .... [302]
126B2        .......... .......... .......... .......... .......... .... [302]
216A         .......... .......... .......... .......... .......... .... [302]
216B         .......... .......... .......... .......... .......... .... [302]
KA           .......... .......... .......... .......... .......... .... [302]
KB           .......... .......... .......... .......... .......... .... [302]
297A         .......... .......... .......... .......... .......... .... [302]
297B         .......... .......... .......... .......... .......... .... [302]
95A          .......... .......... .......... .......... .......... .... [302]
95B          .......... .......... .......... .......... .......... .... [302]
246A         .......... .......... .......... .......... .......... .... [302]
246B         .......... .......... .......... .......... .......... .... [302]
370A         .......... .......... .......... .......... .......... .... [302]
370B         .......... .......... .......... .......... .......... .... [302]
361A         .......... .......... .......... .......... .......... .... [302]
361B         .......... .......... .......... .......... .......... .... [302]
SALPAT       .......... .......... .......... .......... .......... .... [302]
YOELIICS     ISSQLTE..S Q......S.. ...K.K.-V. .Q..N...E. ID.EI.K... .SS. [302]
YONIGCS      MSNQLTE..S Q......S.. ...K.K.-V. .Q..N...E. ID.EI.K... .SS. [302]
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Alignment 5: CSP gene, nucleotide sequences from Manaus and references Salvador (SALPAT) and Belém (BELPAT), and sequences retrieved from Gene Bank. Belém was the reference strain, identical nucleotides are represented by dots, and gaps are represented by dashes.

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BELPAT        AAG GAT GGA AAG AAA GCA GAA CCA AAA AAT CCA CGT GAA AAT AAG CTG AAA CAA CCA  [ 57]
SALPAT        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
YOELII        ..A ..A .A. ..A ... ..T ..T ... CC. ..A GAG GC. C.. ... ..A T.. ... ..G ...  [ 57]
103A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B2         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
243A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
294B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
297A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
297B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95A           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95B           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
A             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
B             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
C             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
D             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
E             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ...  [ 57]
F             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
G             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
H             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
I             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
J             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KA            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KB            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
CH4           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
CH5           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
G24           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP0067      ..A .G. ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP9550      ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP966       ... ... ... ..A ... ... ... ..C ... ... ..C ... ... ... ... ..T ... ... ..G  [ 57]
KCSP967       ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP9674      ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP971       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KCSP9775      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KNIHCSP078099 ..A .G. ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ...  [ 57]
KNIHCSP99056  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KNIHCSP99099  ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KNIHCSP99161  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9611    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9621    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9625    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9633    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9642    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP971     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP9711    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KPVCSP98288   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
NKS           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
NYUTHAI       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
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PH46          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
SOL101        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
SOL83         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
131           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
136           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
185           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
212           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
321           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
340           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
442           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
66            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
738           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
827           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
913           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [ 57]
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Alignment 5 continuing

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BELPAT        --- GGA GAC AGA GCA GAT GGA CAG CCA GCA GGA GAC AGA GCA GAT GGA CAG CCA GCA [114]
SALPAT        --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
YOELII        GCA --- --- GC. .T. .CA .AC .CA AAT ..G CC. .TA GC. .AC CCA AAT GC. ... .T. [114]
103A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
126A          GCA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ... [114]
126B1         --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
126B2         --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
216A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
216B          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
243A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
246A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
246B          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
294B          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
297A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
297B          GCA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ... [114]
361A          --- ..T ... ... ... ... ... ... ... ..T ... ..T ... ..T ... ... ... ... ... [114]
361B          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
370A          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
370B          --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
95A           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
95B           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
A             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... [114]
B             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
C             GCA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ... [114]
D             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
E             GCA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ... [114]
F             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
G             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
H             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
I             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
J             --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
KA            --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
KB            --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
CH4           --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
CH5           --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
G24           ACA ..T ..T ... ... ... ... ... ... ... ... ..T ... ... .C. ... ... ... ... [114]
KCSP0067      --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KCSP9550      --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KCSP966       --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KCSP967       --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KCSP9674      --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ..A ... ... [114]
KCSP971       --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KCSP9775      --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KNIHCSP078099 --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KNIHCSP99056  --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KNIHCSP99099  --- ... ... ... ... ... ... ... ... ... ..T ..T ... ... .C. ... ... ... ... [114]
KNIHCSP99161  --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9611    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9621    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9625    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9633    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9642    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP971     --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP9711    --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
KPVCSP98288   --- ... ... ... ... ... ... ... G.. ... ... A.T G.T ... .G. ... ... ... ... [114]
NKS           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
NYUTHAI       --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [114]
PH46          GGA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ... [114]
```

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SOL101        GCA ..T ..T ... ... ... ... ... ... ... ..T ..T ... ... ... ... ... ... ...  [114]
SOL83         --- .T. ... ... ... ... ... ... ... --- ... ... ... ... ... ... ... ... ...  [114]
131           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
136           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
185           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
212           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
321           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
340           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
442           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
66            --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
738           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
827           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
913           --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
```

Alignment 5 continuing

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BELPAT        GGT GAT AGA GCA GAT GGA CAA CCA GCA GGA GAT AGA GCA GCT GGA CAG CCA GCA GGA  [171]
SALPAT        ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
YOELII        .CA ..C --- --- CCA AAT GCG ... .T. .C. ..C CC. AAT ..G CC. GTA G.. .AC ---  [171]
103A          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
126A          ... ... ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ... ... ... ..T  [171]
126B1         ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ...  [171]
126B2         ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ... ... ... ...  [171]
216A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
216B          ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ...  [171]
243A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
246A          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
246B          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
294B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
297A          ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ...  [171]
297B          ... ... ... ... ... ... ..G ... ... ..T ... ... ... ... ... ..A ... ... ..T  [171]
361A          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ... ... ... ...  [171]
361B          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
370A          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
370B          ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
95A           ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
95B           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
A             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
B             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
C             ... ... ... ... ... ... ..G ... ... ..T ... ... ... ... ... ..A ... ... ..T  [171]
D             ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
E             ... ... ... ... ... ... ..G ... ... ..T ... ... ... ... ... ..A ... ... ..T  [171]
F             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
G             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
H             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
I             ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
J             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [171]
KA            ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
KB            ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
CH4           ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
CH5           ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
G24           ... ... ... ... ... ... ... ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]
KCSP0067      ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP9550      ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP966       ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP967       ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP9674      ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP971       ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KCSP9775      ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KNIHCSP078099 ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KNIHCSP99056  ... ... ... ... .C. ... ... ... ... ..T ... G.. ... ... ... ... ... ... ..C  [171]
KNIHCSP99099  ..C ... G.. ... .C. ... ..G ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KNIHCSP99161  ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP9611    ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP9621    ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP9625    ... ... ... ..T .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP9633    ... ... ... ... ... ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP9642    ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
KPVCSP971     ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
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KPVCSP98288   ... ... ... ... .C. ... ... ... ... ..C ... G.. ... ... ... ... ... ... ..C  [171]
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PH46          ... ... ... ... .C. ... ... ... ... ..T ... ... ... .A. ... ... ... ... ..C  [171]
SOL101        ... ... ... ... .C. ... ... ... ... ..T ... ... ... .A. ... ... ... ... ..C  [171]
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913            ..A ..C ... ... ... ... ..G ... ... ..T ... ... ... .A. ... ..A ... ... ...  [171]


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C              ... ... ... ... ... ... ... ... ... ..C ..T ... ... .C. ... ... ... ..C ..T  [228]
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F              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
G              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
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CH5            ... G.. ... .C. ... ... ... ... ... ..C ..T ... ... .C. ... ..A ... ... ..C ..T  [228]
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Alignment 5 continuing

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361B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ..C ...  [285]
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G24           ... ... ... ... ..A ... ... ... ... ... ... ... ... ..A ... ... ..A ..C ...  [285]
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SOL83         ... ... ... ... ... ... ... ... ..T ... ... .C. ... ... ... ... ..C ... ...  [285]
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913           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [285]
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Alignment 5 continuing

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913          ... .A. ... ... ... ... ..A ... ... ... .A. ... ... ... ... ..A ..C ... ... [342]
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Alignment 5 continuing

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294B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [399]
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297B         ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..T ... ... ... .C.  [399]
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95A          ... ... ... ... ... ... ..C ... ... ... .A. ... ... ... ... ..T ... ... ... ...  [399]
95B          ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ..A ... ... ... .C.  [399]
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CH5          .C. ... ... ... ... ..C ... ... ... ... ... ... ..A ... ... ..T ... ... ... .C.  [399]
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NYUTHAI      ... ... ... ... ... ... ... ... ... ..T --- ... ... ... ... ... ... ... ...  [399]
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136          ... ... ... ... ... ... ..C ... ... .A. ... ... ... ... ..T ... ... ... ...  [399]
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66            .C. ... ... ... ... ..C ... ... ... ... .A. ... ... ... ... ..A ... ... ... .C. [399]
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Alignment 5 continuing

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136           ... ..A ... ... ... ... ... ... .A. ... ..G ... ... ..T ... ... ... ... ... [456]
185           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [456]
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738           ... ..A ... ... ... ... ... ... .A. ... ..G ... ... ... ... ... ... ... ...  [456]
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913           ... ..A ... ... ... ... ... ... .A. ... ..G ... ... ..T ... ... ... ... ...  [456]
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Alignment 5 continuing

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216B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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246B          ..G ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
294B          ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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297B          ..G ... ... ... ... ... ... .A. ... ..A ... ... ... ... ... ... ... ... ...  [513]
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95B           ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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CH5           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
G24           ..G ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ...  [513]
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Alignment 5 continuing

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216B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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361B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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370B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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KB            ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... G..  [570]
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G24           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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KPVCSP9621    G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP9625    G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP9633    G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP9642    G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP971     G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP9711    G.. ... ... A.. G.T ... .G. ... ... G.. ... ... A.. G.T ... .G. ... ... G..  [570]
KPVCSP98288   G.. ... ... A.A G.G ... .G. ... ... G.C ... A.. A.. G.T ... .G. ... ... G..  [570]
NKS           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G..  [570]
NYUTHAI       ... ... ... ... ... ..T --- ... ... ... ... ... ... ... ..T --- ... ... ...  [570]
PH46          ... ... ... A.. G.T ... .G. ... ... G.. ... --- --- --- --- --- --- --- ---  [570]
SOL101        ... ... ..C ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- ---  [570]
SOL83         ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- ---  [570]
131           ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
136           ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
185           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
212           ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [570]
321           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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340           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [570]
442           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [570]
66            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [570]
738           ... ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- [570]
827           ... ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- [570]
913           ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- [570]
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Alignment 5 continuing

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BELPAT        GCA GGA AAT GGT GCA GGT GGA CAG GCA GCA --- --- --- --- --- --- --- --- --- --- --- [627]
SALPAT        ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
YOELII        CA. CC. --- --- --- --- --- --- --- --- GGT GAT AAT AAC AAC AAC AAC AAC AAC AAT  [627]
103A          --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
126A          --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
126B1         ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
126B2         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- [627]
216A          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
216B          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
243A          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
246A          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
246B          --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
294B          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
297A          ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
297B          --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
361A          ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
361B          ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
370A          --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
370B          ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
95A           --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- [627]
95B           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
A             ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
B             ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
C             --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
D             --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
E             --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
F             ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
G             --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
H             ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
I             --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
J             ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
KA            ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
KB            ... ... G.. A.A ... .C. ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
CH4           --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
CH5           --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
G24           ... .C. ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
KCSP0067      --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP9550      --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP966       --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP967       --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP9674      --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP971       ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KCSP9775      --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KNIHCSP078099 --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KNIHCSP99056  ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KNIHCSP99099  --- --- --- --- --- --- --- --- --- --- GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KNIHCSP99161  ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9611    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9621    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9625    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9633    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9642    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP971     ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP9711    ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
KPVCSP98288   ... ... ... ... ... ... ..G .CA ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
NKS           ... ... ... ... ... ... ... ... ... ... GGA GGA AAT GCG GCA AAC AAG AAG GCA  [627]
NYUTHAI       ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- [627]
PH46          --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- [627]
SOL101        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- [627]
SOL83         --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
131           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
136           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
185           ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
212           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
321           ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
340           ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
442           ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- --- --- [627]
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66            ... ... G.. A.A ... .C. ... ... C.. ... --- --- --- --- --- --- --- --- --- [627]
738           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- [627]
827           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- [627]
913           --- ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- [627]
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Alignment 5 continuing

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BELPAT        --- --- --- --- --- --- --- --- --- --- --- --- GGA GGA AAC GCA GGA GGA CAG GGA  [684]
SALPAT        --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
YOELII        AAC AAT AAT --- AAC AAT --- --- --- --- --- --- --- --- ..T AAT --- --- --- ..T  [684]
103A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
126A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
126B1         --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
126B2         --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
216A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
216B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
243A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
246A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
246B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
294B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
297A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
297B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
361A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
361B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
370A          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
370B          --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
95A           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
95B           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
A             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
B             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
C             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
D             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
E             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
F             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
G             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
H             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
I             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
J             --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
KA            --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
KB            --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
CH4           GAA GAC GCA GGA GGA AAC GCA --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
CH5           GAA GAC GCA GGA GGA AAC GCA --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
G24           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
KCSP0067      GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KCSP9550      GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KCSP966       GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KCSP967       GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KCSP9674      GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KCSP971       GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KCSP9775      GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KNIHCSP078099 GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KNIHCSP99056  GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KNIHCSP99099  GAA GAC GCA GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
KNIHCSP99161  GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP9611    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP9621    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP9625    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP9633    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ..T  [684]
KPVCSP9642    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP971     GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP9711    GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
KPVCSP98288   GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
NKS           GAA GAC GCA GGA GGA AAC GCA --- --- --- --- ... ... ... ... ... ... ... ... ...  [684]
NYUTHAI       --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
PH46          --- --- --- GGA GGA AAC GCA GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
SOL101        --- --- --- --- --- --- --- GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
SOL83         --- --- --- --- --- --- --- GGA GGA AAC GCA ... ... ... ... ... ... ... ... ...  [684]
131           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
136           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
185           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
212           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
321           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
340           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
442           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
66            --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
738           --- --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ...  [684]
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827          --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ... [684]
913          --- --- --- --- --- --- --- --- --- --- --- ... ... ... ... ... ... ... ... [684]
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Alignment 5 continuing

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BELPAT       CAA AAT AAT GAA GGT GCG AAT GCC CCA AAT GAA AAG TCT GTG AAA GAA TAC CTA GAT [741]
SALPAT       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
YOELII       A.T ... ..C ... .A. T.T T.. .T. ... .GC .C. G.A CAA A.A TT. ... .TT G.T A.A [741]
103A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
126A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
126B1        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
126B2        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
216A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
216B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
243A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
246A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
246B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
294B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
297A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
297B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
361A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
361B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
370A         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
370B         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
95A          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
95B          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
C            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
D            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
E            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
F            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
G            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
H            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
I            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
J            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KA           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KB           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
CH4          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
CH5          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
G24          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP0067     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP9550     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP966      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP967      ... ... ... ... ... ... ... ... ... .C ... ... ... ... ... ... ... ... ... [741]
KCSP9674     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP971      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KCSP9775     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KNIHCSP078099 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KNIHCSP99056 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KNIHCSP99099 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KNIHCSP99161 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9611   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9621   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9625   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9633   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9642   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP971    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP9711   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
KPVCSP98288  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
NKS          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
NYUTHAI      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
PH46         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
SOL101       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
SOL83        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
131          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
136          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
185          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
212          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
321          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
340          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
442          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
66           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
738          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
827          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
913          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... [741]
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Alignment 5 continuing

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BELPAT          AAA GTT AGA GCT ACC GTT GGC ACC GAA TGG ACT CCA TGC AGT GTA ACC TGT GGA GTG  [798]
SALPAT          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
YOELII          C.G A.G ..C AA. CAA C.C ACA GAG ... ... T.. .A. ..T ... ... ... ... ..T TCT  [798]
103A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126B1           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
126B2           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
216A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
216B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
243A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
246A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
246B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
294B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
297A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
297B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
361A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
361B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
370A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
370B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
95A             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
95B             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
A               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
B               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
C               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
D               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
E               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
F               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
G               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
H               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
I               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
J               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KA              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KB              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
CH4             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
CH5             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
G24             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP0067        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP9550        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP966         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP967         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP9674        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP971         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KCSP9775        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KNIHCSP078099   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KNIHCSP99056    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KNIHCSP99099    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KNIHCSP99161    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9611      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9621      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9625      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9633      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9642      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP971       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP9711      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
KPVCSP98288     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
NKS             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
NYUTHAI         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
PH46            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
SOL101          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
SOL83           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
131             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
136             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
185             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
212             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
321             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
340             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
442             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
66              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
738             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
827             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
913             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [798]
```

Alignment 5 continuing

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BELPAT          GGT GTA AGA GTC AGA AGA AGA GTT AAT GCA GCT AAC AAA AAA CCA GAG GAT CTT ACT  [855]
SALPAT          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
YOELII          ... ... ... ..T ... .A. C.. AAA ... --- .TA ... ..G C.. ... ..A A.. T.G ..C  [855]
103A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
126A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
126B1           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
126B2           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
216A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
216B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
243A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
246A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
246B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
294B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
297A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
297B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
361A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
361B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
370A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
370B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
95A             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
95B             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
A               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
B               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
C               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
D               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
E               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
F               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
G               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
H               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
I               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
J               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KA              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KB              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
CH4             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
CH5             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
G24             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP0067        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP9550        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP966         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP967         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP9674        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP971         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KCSP9775        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KNIHCSP078099   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KNIHCSP99056    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KNIHCSP99099    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KNIHCSP99161    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9611      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9621      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9625      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9633      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9642      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP971       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP9711      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
KPVCSP98288     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
NKS             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
NYUTHAI         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
PH46            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
SOL101          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
SOL83           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
131             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
136             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
185             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
212             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
321             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
340             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
442             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
66              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
738             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
827             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
913             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [855]
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Alignment 5 continuing

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BELPAT          TTG AAT GAC CTT GAG ACT GAT GTT TGT ACA ATG GAT AAG TGT GCT GGC ATA TT [908]
SALPAT          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
YOELII          ..A G.G ..T A.. ..T ... ..A A.. ... .A. ... ... ..A ... T.A A.T ... .. [908]
103A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
126A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
126B1           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
126B2           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
216A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
216B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
243A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
246A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
246B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
294B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
297A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
297B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
361A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
361B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
370A            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
370B            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
95A             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
95B             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
A               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
B               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
C               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
D               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
E               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
F               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
G               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
H               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
I               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
J               ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KA              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KB              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
CH4             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
CH5             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
G24             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP0067        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP9550        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP966         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP967         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP9674        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP971         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KCSP9775        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KNIHCSP078099   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KNIHCSP99056    C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KNIHCSP99099    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KNIHCSP99161    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9611      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9621      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9625      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9633      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9642      ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP971       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP9711      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
KPVCSP98288     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
NKS             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
NYUTHAI         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
PH46            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
SOL101          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
SOL83           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
131             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
136             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
185             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
212             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
321             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
340             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
442             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
66              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
738             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
827             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
913             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [908]
```

Alignment 6: MSP-1 amino acid sequences from Manaus and references Salvador (SALPAT) and Belém (BELEM, BELPAT). Identical amino acids are represented by dots, and gaps are represented by dashes, amino acids unique to strains from Manaus in red.

```
BELEM      IDKLKDFIPK IESMIATEKA KPAASAP--V TSGQLLRGSS EAATEVTTNA VTSEDQQQ [ 58]
BELPAT     .......... .......... .......--. .......... .......... ....V... [ 58]
SALPAT     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
A-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
B-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
C-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
D-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
E-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
F-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
G-HOSP     .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
H-HOSP     .......... .......... .......--. .......... .......... ....V... [ 58]
I-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
J-HOSP     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
KA-HOSP    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
KB-HOSP    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
131-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
136-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
185-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
212-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
321-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
340-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
442-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
66-NOREL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
738-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
827-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
913-NOREL  .......... .......... .......--. .......... .......... ....V... [ 58]
103A-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
103B-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
126A-REL   .......... .......... .......--. .......... .......... ....V... [ 58]
126B-REL   .......... .......... .......--. .......... .......... ....V... [ 58]
216A-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
216B-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
243A-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
246A-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
246B-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
294A-REL   .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
294B-REL   .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
297A-REL   .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
361A-REL   .......... .......... .......--. .......... .......... ....V... [ 58]
361B-REL   .......... .......... .......--. .......... .......... ....V... [ 58]
370A-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
95A-REL    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
95B-REL    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
```

Alignment 6 continuing

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BELEM      QQQQQQQQQQ QQQQQQQQQQ ---------- ----SQVVPA PAGDAQQVIS TQPTSQSA [116]
BELPAT     .......... .......... ---------- ----...... .......... ........ [116]
SALPAT     -.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
A-HOSP     .......... .....---- ---------- ----...... .......... ........ [116]
B-HOSP     ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
C-HOSP     .......... .........- ---------- ----...... .......... ........ [116]
D-HOSP     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T.R.AP [116]
E-HOSP     .......... ......---- ---------- ----...... .......... ........ [116]
F-HOSP     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
G-HOSP     .......... ....------ ---------- ----...... .......... ........ [116]
H-HOSP     .......... ..-------- ---------- ----...... .......... ........ [116]
I-HOSP     ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
J-HOSP     .......... .....---- ---------- ----...... .......... ........ [116]
KA-HOSP    .......... .........- ---------- ----...... .......... ........ [116]
KB-HOSP    .......... .........- ---------- ----...... .......... ........ [116]
131-NOREL  ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
136-NOREL  ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
185-NOREL  .......... .........- ---------- -----L.... .......... ........ [116]
212-NOREL  .......... .....---- ---------- ----...... .......... ........ [116]
321-NOREL  P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
340-NOREL  .......... .........- ---------- -----L.... .......... ........ [116]
442-NOREL  .......... .........- ---------- -----L.... .......... ........ [116]
66-NOREL   .......... ......---- ---------- ----...... .......... ........ [116]
738-NOREL  .......... .........- ---------- ----...... .......... ........ [116]
827-NOREL  .......... .........- ---------- ----...... .......... ........ [116]
913-NOREL  .......... ..-------- ---------- ----...... .......... ........ [116]
103A-REL   .......... .........- ---------- ----...... .......... ........ [116]
103B-REL   .......... .........- ---------- ----...... .......... ........ [116]
126A-REL   .......... .......... QQQQQQQ--- ----...... .......... ........ [116]
126B-REL   .......... .......... QQQQQQQ--- ----...... .......... ........ [116]
216A-REL   P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
216B-REL   P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
243A-REL   .......... .........- ---------- -----L.... .......... ........ [116]
246A-REL   .......... .........- ---------- ----...... .......... ........ [116]
246B-REL   .......... .........- ---------- ----...... .......... ........ [116]
294A-REL   .......... ....------ ---------- ----...... .......... ........ [116]
294B-REL   .......... ....------ ---------- ----...... .......... ........ [116]
297A-REL   .......... ....------ ---------- ----...... .......... ........ [116]
361A-REL   .......... .......... QQQQQQQ--- ----...... .......... ........ [116]
361B-REL   .......... .......... QQQQQQQ--- ----...... .......... ........ [116]
370A-REL   .......... .........- ---------- ----...... .......... ........ [116]
95A-REL    .......... .........- ---------- -----L.... .......... ........ [116]
95B-REL    .......... .........- ---------- ----...... .......... ........ [116]
```

Alignment 6 continuing

```
BELEM      APGVSAT--- ---PAPTPAA AAAPAPAMSK LEYLEKLLDF LKSAYACHKH IFVTNSTM [174]
BELPAT     .......... ---.....  .........  .........  .........  ........ [174]
SALPAT     ..TQASQEPA PAA.PS....  .V....T...  .........  .........  ........ [174]
A-HOSP     .......... ---.....  .........  .........  .........  ........ [174]
B-HOSP     ..TQASQEPA PAA.PS....  .V....T...  ....Q.....  .........  ........ [174]
C-HOSP     .......... ---.....  .........  .........  .........  ........ [174]
D-HOSP     ..TQASQEPA PAV.PS....  .V....T...  ....Q.....  .........  ........ [174]
E-HOSP     .......... ---.....  .........  .........  .........  ........ [174]
F-HOSP     ..TQASQEPA PAV.PS....  .V....T...  ....Q.....  .........  ........ [174]
G-HOSP     ..D....... ---.....  .........  .........  .........  ........ [174]
H-HOSP     .......... ---.....  .........  .........  .........  ........ [174]
I-HOSP     ..TQASQEPA PAA.PS....  .V....T...  ....Q.....  .........  ........ [174]
J-HOSP     .......... ---.....  .........  .........  .........  ........ [174]
KA-HOSP    .......... ---.....  .........  .........  .........  ........ [174]
KB-HOSP    .......... ---.....  .........  .........  .........  ........ [174]
131-NOREL  ..TQASQEPA PAA.PS....  .V....T...  ....Q.....  .........  ........ [174]
136-NOREL  ..TQASQEPA PAA.PS....  .V....T...  ....Q.....  .........  ........ [174]
185-NOREL  ..D....... ---.....  .........  .........  .........  ........ [174]
212-NOREL  .......... ---.....  .........  .........  .........  ........ [174]
321-NOREL  ..TQASQEPA PAV.PS....  .V....T...  ....Q.....  .........  ........ [174]
340-NOREL  ..D....... ---.....  .........  .........  .........  ........ [174]
442-NOREL  ..D....... ---.....  .........  .........  .........  ........ [174]
66-NOREL   .......... ---.....  .........  .........  .........  ........ [174]
738-NOREL  .......... ---.....  .........  .........  .........  ........ [174]
827-NOREL  .......... ---.....  .........  .........  .........  ........ [174]
913-NOREL  .......... ---.....  .........  .........  .........  ........ [174]
103A-REL   .......... ---.....  .........  .........  .........  ........ [174]
103B-REL   .......... ---.....  .........  .........  .........  ........ [174]
126A-REL   .......... ---.....  .........  .........  .........  ........ [174]
126B-REL   .......... ---.....  .........  .........  .........  ........ [174]
216A-REL   ..TQASQEPA PAV.PS....  .V....T...  ....Q.....  .........  ........ [174]
216B-REL   ..TQASPEPA PAV.PS....  .V....T...  ....Q.....  .........  ........ [174]
243A-REL   ..D....... ---.....  .........  .........  .........  ........ [174]
246A-REL   .......... ---.....  .........  .........  .........  ........ [174]
246B-REL   .......... ---.....  .........  .........  .........  ........ [174]
294A-REL   ..D....... ---.....  .........  .........  .........  ........ [174]
294B-REL   ..D....... ---.....  .........  .........  .........  ........ [174]
297A-REL   ..D....... ---.....  .........  .........  .........  ........ [174]
361A-REL   .......... ---.....  .........  .........  .........  ........ [174]
361B-REL   .......... ---.....  .........  .........  .........  ........ [174]
370A-REL   .......... ---.....  .........  .........  .........  ........ [174]
95A-REL    ..D....... ---.....  .........  .........  .........  ........ [174]
95B-REL    .......... ---.....  .........  .........  .........  ........ [174]
```

Alignment 6 continuing

```
BELEM      DKKLLKEYEL NADEKTKINQ NKCDELDLLF NVQNNLPAMY SIYDSMSN [222]
BELPAT     .......... .......... .......... .......... ........ [222]
SALPAT     K.E..DQ.K. ....QN...E T......... .......... ........ [222]
A-HOSP     .......... .......... .......... .......... ........ [222]
B-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
C-HOSP     .......... .......... .......... .......... ........ [222]
D-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
E-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
F-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
G-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
H-HOSP     .......... .......... .......... .......... ........ [222]
I-HOSP     K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
J-HOSP     .......... .......... .......... .......... ........ [222]
KA-HOSP    .......... .......... .......... .......... ........ [222]
KB-HOSP    .......... .......... .......... .......... ........ [222]
131-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
136-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
185-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
212-NOREL  .......... .......... .......... .......... ........ [222]
321-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
340-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
442-NOREL  K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
66-NOREL   .......... .......... .......... .......... ........ [222]
738-NOREL  .......... .......... .......... .......... ........ [222]
827-NOREL  .......... .......... .......... .......... ........ [222]
913-NOREL  .......... .......... .......... .......... ........ [222]
103A-REL   .......... .......... .......... ......A... ........ [222]
103B-REL   .......... .......... .......... .......... ........ [222]
126A-REL   .......... .......... .......... .......... ........ [222]
126B-REL   .......... .......... .......... .......... ........ [222]
216A-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
216B-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
243A-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
246A-REL   .......... .......... .......... ......A... ........ [222]
246B-REL   .......... .......... .......... .......... ........ [222]
294A-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
294B-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
297A-REL   K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
361A-REL   ...P...... .......... .......... .......... ........ [222]
361B-REL   .......... .......... .......... .......... ........ [222]
370A-REL   .......... .......... .......... .......... ........ [222]
95A-REL    K.E..DQ.K. ....QN..KE .......... .......... ........ [222]
95B-REL    .......... .......... .......... .......... ........ [222]
```

Alignment 7: MSP-1 gene, nucleotide sequences from Manaus and reference sequences Salvador I (SALPAT) and Belém (BELEM, BELPAT). Belém was the reference strain, identical nucleotides are represented by dots, and gaps are represented by dashes.

```
BELEM       ATT GAC AAG TTG AAG GAC TTC ATC CCC AAA ATC GAG AGC ATG ATC GCC ACT GAG AAG  [ 57]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
B-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
D-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
E-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
F-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
G-HOSP      ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
I-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
131-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
136-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
321-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
294A-REL    ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
294B-REL    ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
297A-REL    ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95A-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SALPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
```

Alignment 7 continuing

```
BELEM       GCC AAG CCG GCA GCG TCA GCG CCA --- --- GTG ACC AGT GGA CAA TTG CTT AGA GGA  [114]
BELPAT      ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
A-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
B-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
C-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
D-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
E-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
F-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
G-HOSP      ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
H-HOSP      ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
I-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
J-HOSP      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
KA-HOSP     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
KB-HOSP     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
131-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
136-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
185-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
212-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
321-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
340-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
442-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
66-NOREL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
738-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
827-NOREL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
913-NOREL   ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
103A-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
103B-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
126A-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
126B-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
216A-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
216B-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
243A-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
246A-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
246B-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
294A-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
294B-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
297A-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
361A-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
361B-REL    ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
370A-REL    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
95A-REL     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
95B-REL     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SALPAT      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
```

Alignment 7 continuing

```
BELEM       TCA AGC GAA GCA GCG ACA GAG GTC ACA ACC AAT GCG GTA ACA TCT GAA GAT CAA CAA  [171]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
A-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
B-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
C-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
D-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
E-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
F-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
G-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
I-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
J-HOSP      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
KA-HOSP     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
KB-HOSP     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
131-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
136-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
185-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
212-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
321-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
340-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
442-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
66-NOREL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
738-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
827-NOREL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
103A-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
103B-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
216A-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
216B-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
243A-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
246A-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
246B-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
294A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
370A-REL    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
95A-REL     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
95B-REL     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SALPAT      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
```

Alignment 7 continuing

```
BELEM       CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAG CAA CAA CAG CAA CAA  [228]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
A-HOSP      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
B-HOSP      ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
C-HOSP      ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
D-HOSP      .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
E-HOSP      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
F-HOSP      .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
G-HOSP      ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
H-HOSP      ... ... ... ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- ---  [228]
I-HOSP      ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
J-HOSP      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
KA-HOSP     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
KB-HOSP     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
131-NOREL   ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
136-NOREL   ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
185-NOREL   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
212-NOREL   ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
321-NOREL   .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
340-NOREL   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
442-NOREL   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
66-NOREL    ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
738-NOREL   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
827-NOREL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
913-NOREL   ... ... ... ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- ---  [228]
103A-REL    ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
103B-REL    ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
216A-REL    .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
216B-REL    .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
243A-REL    ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
246A-REL    ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
246B-REL    ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
294A-REL    ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
294B-REL    ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
297A-REL    ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
370A-REL    ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
95A-REL     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
95B-REL     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
SALPAT      .C. --- ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
```

Alignment 7 continuing

```
BELEM       CAA CAA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- TCA CAA GTA  [285]
BELPAT      ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
A-HOSP      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
B-HOSP      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
C-HOSP      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
D-HOSP      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
E-HOSP      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
F-HOSP      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
G-HOSP      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
H-HOSP      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
I-HOSP      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
J-HOSP      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
KA-HOSP     ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
KB-HOSP     ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
131-NOREL   ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
136-NOREL   ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
185-NOREL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
212-NOREL   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
321-NOREL   ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
340-NOREL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
442-NOREL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
66-NOREL    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
738-NOREL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
827-NOREL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
913-NOREL   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
103A-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
103B-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
126A-REL    ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- ... ... ...  [285]
126B-REL    ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- ... ... ...  [285]
216A-REL    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
216B-REL    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
243A-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
246A-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
246B-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
294A-REL    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
294B-REL    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
297A-REL    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
361A-REL    ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- ... ... ...  [285]
361B-REL    ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- ... ... ...  [285]
370A-REL    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
95A-REL     ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
95B-REL     ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
SALPAT      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
```

Alignment 7 continuing

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BELEM       GTA CCA GCA CCT GCA GGA GAT GCC CAA CAA GTA ATC TCA ACA CAA CCG ACT AGT CAA  [342]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
B-HOSP      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
D-HOSP      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..G ...  [342]
E-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
F-HOSP      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
G-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
I-HOSP      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
131-NOREL   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
136-NOREL   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
321-NOREL   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
216A-REL    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
216B-REL    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
294A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
95A-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
SALPAT      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
```

Alignment 7 continuing

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BELEM       TCC GCA GCA CCA GGC GTA TCA GCC ACA --- --- --- --- --- --- CCA GCA CCA ACA  [399]
BELPAT      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
A-HOSP      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
B-HOSP      G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GCC ... C.. T.G ...  [399]
C-HOSP      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
D-HOSP      G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GTC ... C.. T.G ...  [399]
E-HOSP      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
F-HOSP      G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GTC ... C.. T.G ...  [399]
G-HOSP      ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
H-HOSP      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
I-HOSP      G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GCC ... C.. T.G ...  [399]
J-HOSP      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
KA-HOSP     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
KB-HOSP     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
131-NOREL   G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GCC ... C.. T.G ...  [399]
136-NOREL   G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GCC ... C.. T.G ...  [399]
185-NOREL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
212-NOREL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
321-NOREL   G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GTC ... C.. T.G ...  [399]
340-NOREL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
442-NOREL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
66-NOREL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
738-NOREL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
827-NOREL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
913-NOREL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
103A-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
103B-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
126A-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
126B-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
216A-REL    G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GTC ... C.. T.G ...  [399]
216B-REL    G.. C.. ... ... ACT CA. G.C T.. C.. GAA CCA GCA CCA GCC GTC ... C.. T.G ...  [399]
243A-REL    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
246A-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
246B-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
294A-REL    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
294B-REL    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
297A-REL    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
361A-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
361B-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
370A-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
95A-REL     ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
95B-REL     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- ... ... ... ...  [399]
SALPAT      G.. C.. ... ... ACT CA. G.C T.. CA. GAA CCA GCA CCA GCC GCC ... C.. T.G ...  [399]
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Alignment 7 continuing

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BELEM       CCT GCT GCC GCA GCC GCC CCA GCA CCA GCC ATG TCC AAA CTG GAA TAC CTC GAA AAG  [456]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
B-HOSP      ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
D-HOSP      ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
E-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
F-HOSP      ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
G-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
I-HOSP      ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
131-NOREL   ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
136-NOREL   ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
185-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
321-NOREL   ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
340-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
442-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
216A-REL    ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
216B-REL    ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ..T ..G C.. ...  [456]
243A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
294A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
294B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
297A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
95A-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
SALPAT      ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ... ... ... ...  [456]
```

Alignment 7 continuing

BELEM	CTC	CTT	GAT	TTT	TTA	AAA	TCC	GCT	TAC	GCA	TGT	CAC	AAG	CAC	ATC	TTC	GTA	ACC	AAC	[513]
BELPAT	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
A-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
B-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
C-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
D-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
E-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
F-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
G-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
H-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
I-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
J-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
KA-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
KB-HOSP	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
131-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
136-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
185-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
212-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
321-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
340-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
442-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
66-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
738-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
827-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
913-NOREL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
103A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
103B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
126A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
126B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
216A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
216B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
243A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
246A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
246B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
294A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
294B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
297A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
361A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
361B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
370A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
95A-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	..G	...	...	[513]
95B-REL	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	[513]
SALPAT	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..T	...	...	...	[513]

Alignment 7 continuing

```
BELEM       TCC ACC ATG GAC AAG AAA CTA CTC AAA GAG TAC GAA CTT AAC GCT GAT GAG AAA ACC  [570]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
B-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
D-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
E-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
F-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
G-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
I-HOSP      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
131-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
136-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
185-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
321-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
340-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
442-NOREL   ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
216A-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
216B-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
243A-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
246A-REL    ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ...  [570]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
294A-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
294B-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
297A-REL    ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
361A-REL    ... ... ... ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ...  [570]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
95A-REL     ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
SALPAT      ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... ... ... C.. .A.  [570]
```

Alignment 7 continuing

```
BELEM       AAA ATT AAT CAA AAC AAA TGC GAT GAA TTG GAC CTC CTA TTC AAT GTC CAG AAC AAC  [627]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
B-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
D-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
E-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
F-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
G-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
I-HOSP      ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
131-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
136-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
185-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
321-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
340-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
442-NOREL   ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
103A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
126A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
126B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
216A-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
216B-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
243A-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
246A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
294A-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
294B-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
297A-REL    ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
361A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
361B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
95A-REL     ... ... ..G G.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...  [627]
SALPAT      ... ... ..C G.. .CT ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [627]
```

Alignment 7 continuing

```
BELEM       TTG CCA GCC ATG TAC TCC ATA TAT GAC TCC ATG AGC AAC GA [668]
BELPAT      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
A-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
B-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
C-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
D-HOSP      ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
E-HOSP      ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
F-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
G-HOSP      ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
H-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
I-HOSP      ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
J-HOSP      ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
KA-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
KB-HOSP     ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
131-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
136-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
185-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
212-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
321-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
340-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
442-NOREL   ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
66-NOREL    ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
738-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
827-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
913-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
103A-REL    ... G.. ... ... ... ... ... ... ... ... ... ... ... .. [668]
103B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
126A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
126B-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
216A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
216B-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
243A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
246A-REL    ... G.. ... ... ... ... ... ... ... ... ... ... ... .. [668]
246B-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
294A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
294B-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
297A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
361A-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
361B-REL    ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
370A-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
95A-REL     ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
95B-REL     ... ... ... ... ... ... ... ... ... ... ... ... ... .. [668]
SALPAT      ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [668]
```

Alignment 8: MSP-1 amino acid sequences from Manaus, reference sequences Belém (BELEM, BELPAT) and Salvador I (SALPAT), and sequences retrieved from Gene Bank. Identical amino acids represented by dots and gaps represented by dashes.

```
BELEM-BEL   IDKLKDFIPK IESMIATEKA KPAASAP--V TSGQLLRGSS EAATEVTTNA VTSEDQQQ [ 58]
BELPAT-BEL  .......... .......... .......--. .......... .......... ....V... [ 58]
BD1-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BD2-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BD4-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
BD6-GB      .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
BD9-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BP1-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BP13-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
BP29-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BP30-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BP39-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
BP63-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
BR07-GB     .......... .......... .......--. .......... .......... ....V... [ 58]
BR44-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
IN1-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
ITA1-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
ITA2-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
ITA3-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
ITA4-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
PUNJ14-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
PUNJ16-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
PUNJ23-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
PUNJ24-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
SK1-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
SK2-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
SK3-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
SK4-GB      .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
T064-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
T077-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
T107-GB     .......... .QA..T.... .......--. .......... .......... ....V... [ 58]
T117-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
T124-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
T128-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
T131-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TC103-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TC22-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TC28-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TD183-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TD201-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TD207-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD29-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD297A-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TD403-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD414-GB    .......... .........T ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.H [ 58]
TD424-GB    .......... .........D ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD425-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD425B-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD430A-GB   .......... .........T ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD430B-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TD438-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.H [ 58]
TD439A-GB   .......... .......... ..T--.---. .......... .R........ ....V... [ 58]
TD439B-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD452-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TD458A-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
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TD458B-GB   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TE26-GB     .......... .QA..T.... .........-. .......... .......... ....V... [ 58]
TF127-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TF14-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TFF18-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TG40-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TG44-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TG46-GB     .......... .......... .........-. .......... .........G ....V... [ 58]
TG48-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TG55-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
TG57-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TUR1-GB     .AV....... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
TUR2-GB     .......... .......... .........-. .......... .......... ....V... [ 58]
TUR3-GB     .......... .......... .........-. .......... .......... ....V... [ 58]
TUR4-GB     .......... .......... .........-. .......... .......... ....V... [ 58]
TUR5-GB     .......... .......... .........-. .......... .......... ....V... [ 58]
TUR7-GB     .......... .......... .........-. .......... .......... ....V... [ 58]
TUR8-GB     .A........ .......... .........-. .......... .......... ....V... [ 58]
TV400-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
V100-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
VM278-GB    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
VM55-GB     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
A-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
B-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
C-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
D-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
E-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
F-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
G-P-ADM     .......... .QA..T.... .........-. .......... .......... ....V... [ 58]
H-P-ADM     .......... .......... .........-. .......... .......... ....V... [ 58]
I-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
J-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
K-P-ADM     .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
KB-P-ADM    .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
131-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
136-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
185-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
212-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
321-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
340-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
442-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
66-P-NOREL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
738-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
827-P-NOREL .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
913-P-NOREL .......... .......... .........-. .......... .......... ....V... [ 58]
103A-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
103B-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
126A-P-REL  .......... .......... .........-. .......... .......... ....V... [ 58]
126B-P-REL  .......... .......... .........-. .......... .......... ....V... [ 58]
216A-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
216B-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.P [ 58]
243A-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
246A-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
246B-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
294A-P-REL  .......... .QA..T.... .........-. .......... .......... ....V... [ 58]
294B-P-REL  .......... .QA..T.... .........-. .......... .......... ....V... [ 58]
297A-P-REL  .......... .QA..T.... .........-. .......... .......... ....V... [ 58]
361A-P-REL  .......... .......... .........-. .......... .......... ....V... [ 58]
361B-P-REL  .......... .......... .........-. .......... .......... ....V... [ 58]
370A-P-REL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
95A-P-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
95B-P-REL   .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.. [ 58]
SALPAT-SAL  .......... .........N ..TVA.ADI. AK..S...A. .TG.TGN.VN AQTAVV.- [ 58]
YOELII-YO   V.R.AQ.... ......K..E RMEQGPAITG E.EEVPS.P. AESSTDRSTQ SSTSSSSS [ 58]
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Alignment 8 continuing

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BELEM-BEL   QQQQQQQQQQ QQQQQQQQQQ ---------- ----SQVVPA PAGDAQQVIS TQPTSQSA [116]
BELPAT-BEL  .......... .......... ---------- ---------- .......... ........ [116]
BD1-GB      ....VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BD2-GB      ....VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BD4-GB      P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BD6-GB      .......... ..-------- ---------- ---------- .......... ........ [116]
BD9-GB      ....VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BP1-GB      P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BP13-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BP29-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BP30-GB     ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
BP39-GB     .......... .........- ---------- -----.L... .......... ........ [116]
BP63-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
BR07-GB     .......... ..-------- ---------- ----...... .......... ........ [116]
BR44-GB     .......... .........- ---------- -----.L... .......... ........ [116]
IN1-GB      P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
ITA1-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
ITA2-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
ITA3-GB     .......... ..-------- ---------- ----...... .......... ........ [116]
ITA4-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
PUNJ14-GB   P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
PUNJ16-GB   P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
PUNJ23-GB   P.H.VANAVT V.PGTTGH.A QGGEAETHTN SVQAA..QQT ...AGG..A. ..T...AP [116]
PUNJ24-GB   P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
SK1-GB      .........-- ---------- ---------- ----...... .......... ........ [116]
SK2-GB      .........-- ---------- ---------- ----...... .......... ........ [116]
SK3-GB      .........-- ---------- ---------- ----...... .......... ........ [116]
SK4-GB      .........-- ---------- ---------- ----...... .......... ........ [116]
T064-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
T077-GB     ....VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AS [116]
T107-GB     .......... .........- ---------- ----...... .......... ........ [116]
T117-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
T124-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT .......... ........ [116]
T128-GB     ..H.VANAVT V.PGTTGH.A QGGEAEAQTN SVQAA..QQT ...AGG..A. ..TII.AP [116]
T131-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT .......... ........ [116]
TC103-GB    P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TC22-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TC28-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TD183-GB    P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TD201-GB    P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TD207-GB    .......... .........- ---------- ----...... .......... ........ [116]
TD29-GB     .......... ..-------- ---------- ----...... .......... ........ [116]
TD297A-GB   P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TD403-GB    .......... ..-------- ---------- ----...... .......... ........ [116]
TD414-GB    P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T..... [116]
TD424-GB    .......... ...------- ---------- ----...... .......... ........ [116]
TD425-GB    .......... ...------- ---------- ----...... .......... ........ [116]
TD425B-GB   .......... ..-------- ---------- ----...... .......... ........ [116]
TD430A-GB   .......... ..-------- ---------- ----...... .......... ........ [116]
TD430B-GB   P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TD438-GB    P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TD439A-GB   .........- ---------- ---------- ----...... .......... ........ [116]
TD439B-GB   .......... .........- ---------- ----...... .......... ........ [116]
TD452-GB    P......... ..-------- ---------- ----...... .......... ........ [116]
TD458A-GB   P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TD458B-GB   .......... ..-------- ---------- ----...... .......... ........ [116]
TE26-GB     .......... .......... ---------- ----...... .......... ........ [116]
TF127-GB    P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TF14-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TFF18-GB    P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
TG40-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT .......... ........ [116]
TG44-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TG46-GB     .......... ..-------- ---------- ----...... .......... ........ [116]
TG48-GB     ....VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AS [116]
TG55-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TII.AP [116]
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TG57-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
TUR1-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR2-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR3-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR4-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR5-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR7-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TUR8-GB     .......... .......... ---------- ---.....T ..........T ........ [116]
TV400-GB    P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
V100-GB     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
VM278-GB    .......... .......... ---------- ---.....T ..........T ........ [116]
VM55-GB     P.H.VANAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
A-P-ADM     .......... .......... ---------- ---.....T ..........T ........ [116]
B-P-ADM     ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
C-P-ADM     .......... .........- ---------- ---.....T ..........T ........ [116]
D-P-ADM     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T.R.AP [116]
E-P-ADM     .......... .......... ---------- ---.....T ..........T ........ [116]
F-P-ADM     P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
G-P-ADM     .......... .......... ---------- ---.....T ..........T ........ [116]
H-P-ADM     .......... .......... ---------- ---.....T ..........T ........ [116]
I-P-ADM     ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
J-P-ADM     .......... .......... ---------- ---.....T ..........T ........ [116]
K-P-ADM     .......... .........- ---------- ---.....T ..........T ........ [116]
KB-P-ADM    .......... .........- ---------- ---.....T ..........T ........ [116]
131-P-NOREL ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
136-P-NOREL ..H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
185-P-NOREL .......... .........- ---------- ----.-L... ..........T ........ [116]
212-P-NOREL .......... .......... ---------- ---.....T ..........T ........ [116]
321-P-NOREL P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
340-P-NOREL .......... .........- ---------- -----.-L... ..........T ........ [116]
442-P-NOREL .......... .........- ---------- -----.-L... ..........T ........ [116]
66-P-NOREL  .......... .......... ---------- ---.....T ..........T ........ [116]
738-P-NOREL .......... .........- ---------- ---.....T ..........T ........ [116]
827-P-NOREL .......... .........- ---------- ---.....T ..........T ........ [116]
913-P-NOREL .......... .......... ---------- ---.....T ..........T ........ [116]
103A-P-REL  .......... .........- ---------- ---.....T ..........T ........ [116]
103B-P-REL  .......... .........- ---------- ---.....T ..........T ........ [116]
126A-P-REL  .......... .......... QQQQQQQ--- ---.....T ..........T ........ [116]
126B-P-REL  .......... .......... QQQQQQQ--- ---.....T ..........T ........ [116]
216A-P-REL  P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
216B-P-REL  P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..T...AP [116]
243A-P-REL  .......... .........- ---------- -----.-L... ..........T ........ [116]
246A-P-REL  .......... .........- ---------- ---.....T ..........T ........ [116]
246B-P-REL  .......... .........- ---------- ---.....T ..........T ........ [116]
294A-P-REL  .......... .......... ---------- ---.....T ..........T ........ [116]
294B-P-REL  .......... .......... ---------- ---.....T ..........T ........ [116]
297A-P-REL  .......... .......... ---------- ---.....T ..........T ........ [116]
361A-P-REL  .......... .......... QQQQQQQ--- ---.....T ..........T ........ [116]
361B-P-REL  .......... .......... QQQQQQQ--- ---.....T ..........T ........ [116]
370A-P-REL  .......... .........- ---------- ---.....T ..........T ........ [116]
95A-P-REL   .......... .........- ---------- -----.-L... ..........T ........ [116]
95B-P-REL   .......... .........- ---------- ---.....T ..........T ........ [116]
SALPAT-SAL  P.H.VVNAVT V.PGTTGH.A QGGEAETQTN SVQAA..QQT ...AGG..A. ..TI..AP [116]
YOELII-YO   SSSTPAAAES SSATLPEA-- ---------- --------.. ..EA.SPSTE ASEETTIP [116]
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Alignment 8 continuing

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BELEM-BEL   APGVSAT--- -------PAP TPAAAAAPAP AMSKLEYLEK LLDFLKSAYA CHKHIFVT [174]
BELPAT-BEL  .......... --------.. .......... .......... .......... ........ [174]
BD1-GB      ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
BD2-GB      ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
BD4-GB      ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
BD6-GB      .......... --------.. .......... .......... .......... ........ [174]
BD9-GB      ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
BP1-GB      ..TQAS-PEP APAV---.PS .....V.... T......... .......... ........ [174]
BP13-GB     ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
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BP29-GB     ..TQAS-PEP APAV---.PS .....V.... T......... ......... ........ [174]
BP30-GB     ..TQAS-PEP APAA---.PS .....V.... T.......Q. ......... ........ [174]
BP39-GB     .......... ........... .......... .......... ......... ........ [174]
BP63-GB     ..TQAS-PEP APAV---.PS .....V.... T.......Q. ......... ........ [174]
BR07-GB     .......... ........... .......... .......... ......... ........ [174]
BR44-GB     .......... ........... .......... .......... ......... ........ [174]
IN1-GB      ..TQAS-PEP APAA---.PS .....V.... .......... ......... ........ [174]
ITA1-GB     ..TQAS-PEP APAV---.PS .....V.... T.P.....Q. ......... ........ [174]
ITA2-GB     ..TQAS-PEP APAV---.PS .....V.... T.......Q. ......... ........ [174]
ITA3-GB     .......... ........... .......... .......... ......... ........ [174]
ITA4-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
PUNJ14-GB   ..TQAS-PEP APAV---.PS .....V.... T......... ......... ........ [174]
PUNJ16-GB   ..TQAS-PEP APAV---.PS .....V.... PL...G..Q. ..C...... ........ [174]
PUNJ23-GB   ..TQAS-PEP APAV---.PS .....V.... .......... V........ ........ [174]
PUNJ24-GB   ..TQAS-PEP APAV---.PS .....V.... T.......Q. V........ ........ [174]
SK1-GB      .......... ........... .......... .......... ......... ........ [174]
SK2-GB      .......... ........... .......... .......... ......... ........ [174]
SK3-GB      .......... ........... .......... .......... ......... ........ [174]
SK4-GB      .......... ........... .......... .......... ......... ........ [174]
T064-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
T077-GB     ..TQAS-PEP APAV---.PS .....V.... T.......Q. ......... ........ [174]
T107-GB     .......... ........... .......... .......... ......... ........ [174]
T117-GB     ..TQAS-PEP APAV---.PS .....V.... .......... ......... ........ [174]
T124-GB     .......... ........... .......... .......... ......... ........ [174]
T128-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
T131-GB     .......... ........... .......... .......... ......... ........ [174]
TC103-GB    ..TQAS-PEP APAV---.PS .....V.... ........Q. ......... ........ [174]
TC22-GB     ..NQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TC28-GB     ..TQAS-PEP APAV---.PS .....V.... ........Q. ......... ........ [174]
TD183-GB    ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD201-GB    ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD207-GB    .......PAP TPAAAAA... .......... .......... ......... ........ [174]
TD29-GB     .......... ........... .......... .......... ......... ........ [174]
TD297A-GB   ..NQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD403-GB    .......... ........... .......... .......... ......... ........ [174]
TD414-GB    .......... ........... .....V.... T......... ......... ........ [174]
TD424-GB    ..D....... ........... .......... .......... ......... ........ [174]
TD425-GB    ..D....... ........... .......... .......... ......... ........ [174]
TD425B-GB   .......... ........... .......... .......... ......... ........ [174]
TD430A-GB   .......... ........... .......... .......... ......... ........ [174]
TD430B-GB   ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD438-GB    ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD439A-GB   .......... ........... .......... .......... ......... ........ [174]
TD439B-GB   .......PAP TPAAAAA... .......... .......... ......... ........ [174]
TD452-GB    .......... ........... .......... .......... ......... ........ [174]
TD458A-GB   ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TD458B-GB   .......... ........... .......... .......... ......... ........ [174]
TE26-GB     .......... ........... .......... .......... ......... ........ [174]
TF127-GB    ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TF14-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TFF18-GB    ..TQAS-PEP APAA---.PS .....V.... T.......Q. ......... ........ [174]
TG40-GB     .......... ........... .......... .......... ......... ........ [174]
TG44-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TG46-GB     .......... ........... .......... .......... ......... ........ [174]
TG48-GB     ..TQAS-PEP APAV---.PS .....V.... T.......Q. ......... ........ [174]
TG55-GB     ..NQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TG57-GB     ..NQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
TUR1-GB     .......... ........... .......... .......... .......... ....F... [174]
TUR2-GB     .......... ........... .......... .......... ......... ........ [174]
TUR3-GB     .......... ........... .......... .......... .......... ....F... [174]
TUR4-GB     .......... ........... ....V..... .......... ......... ........ [174]
TUR5-GB     .......... ........... .......... .......... ......... ........ [174]
TUR7-GB     .......... ........... .......... .......... ......... ........ [174]
TUR8-GB     .......... ........... .......... .......... ......... ........ [174]
TV400-GB    ..NQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
V100-GB     ..TQAS-PEP APAA---.PS .....V.... T......... ......... ........ [174]
VM278-GB    .......... ........... .....V.... T.......Q. ......... ........ [174]
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VM55-GB     ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
A-P-ADM     .......... ........... .......... .......... .......... ........ [174]
B-P-ADM     ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
C-P-ADM     .......... ........... .......... .......... .......... ........ [174]
D-P-ADM     ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
E-P-ADM     .......... ........... .......... .......... .......... ........ [174]
F-P-ADM     ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
G-P-ADM     ..D....... ........... .......... .......... .......... ........ [174]
H-P-ADM     .......... ........... .......... .......... .......... ........ [174]
I-P-ADM     ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
J-P-ADM     .......... ........... .......... .......... .......... ........ [174]
K-P-ADM     .......... ........... .......... .......... .......... ........ [174]
KB-P-ADM    .......... ........... .......... .......... .......... ........ [174]
131-P-NOREL ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
136-P-NOREL ..TQAS-PEP APAA---.PS .....V.... T.......Q. .......... ........ [174]
185-P-NOREL ..D....... ........... .......... .......... .......... ........ [174]
212-P-NOREL .......... ........... .......... .......... .......... ........ [174]
321-P-NOREL ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
340-P-NOREL ..D....... ........... .......... .......... .......... ........ [174]
442-P-NOREL ..D....... ........... .......... .......... .......... ........ [174]
66-P-NOREL  .......... ........... .......... .......... .......... ........ [174]
738-P-NOREL .......... ........... .......... .......... .......... ........ [174]
827-P-NOREL .......... ........... .......... .......... .......... ........ [174]
913-P-NOREL .......... ........... .......... .......... .......... ........ [174]
103A-P-REL  .......... ........... .......... .......... .......... ........ [174]
103B-P-REL  .......... ........... .......... .......... .......... ........ [174]
126A-P-REL  .......... ........... .......... .......... .......... ........ [174]
126B-P-REL  .......... ........... .......... .......... .......... ........ [174]
216A-P-REL  ..TQAS-QEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
216B-P-REL  ..TQAS-PEP APAV---.PS .....V.... T.......Q. .......... ........ [174]
243A-P-REL  ..D....... ........... .......... .......... .......... ........ [174]
246A-P-REL  .......... ........... .......... .......... .......... ........ [174]
246B-P-REL  .......... ........... .......... .......... .......... ........ [174]
294A-P-REL  ..D....... ........... .......... .......... .......... ........ [174]
294B-P-REL  ..D....... ........... .......... .......... .......... ........ [174]
297A-P-REL  ..D....... ........... .......... .......... .......... ........ [174]
361A-P-REL  .......... ........... .......... .......... .......... ........ [174]
361B-P-REL  .......... ........... .......... .......... .......... ........ [174]
370A-P-REL  .......... ........... .......... .......... .......... ........ [174]
95A-P-REL   ..D....... ........... .......... .......... .......... ........ [174]
95B-P-REL   .......... ........... .......... .......... .......... ........ [174]
SALPAT-SAL  ..TQAS-PEP APAA---.PS .....V.... T......... .......... ........ [174]
YOELII-YO   PTTQETQ--- -------.SQ AASSTTPAK. V.T..Y.... .QK..VFS.S ...YVLLQ [174]
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Alignment 8 continuing

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BELEM-BEL   NSTMDKKLLK EYELNADEKT KINQNKCDEL DLLFNVQNNL PAMYSIYDSM SN [226]
BELPAT-BEL  .......... .......... .......... .......... .......... .. [226]
BD1-GB      ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
BD2-GB      ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
BD4-GB      ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
BD6-GB      .......... .......... ...ET..... .......... .......... .S [226]
BD9-GB      ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
BP1-GB      .......... .......... .......... .......... .......... .. [226]
BP13-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
BP29-GB     .......... .......... .......... .......... .......... .. [226]
BP30-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
BP39-GB     .......... .......... .......... .......... .......... .. [226]
BP63-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
BR07-GB     .......... .......... .......... .......... .......... .. [226]
BR44-GB     .......... .......... .......... .......... .......... .. [226]
IN1-GB      .......... .......... .......... .......... .......... .. [226]
ITA1-GB     .......... ........QN .......... .......... .......... .. [226]
ITA2-GB     .......... ........QN .......... .......... .......... .. [226]
ITA3-GB     .......... ........I. .......... .......... .......... .. [226]
ITA4-GB     ....K.E..D Q.K.....QN ...ET.WH.. .......... .......... .. [226]
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PUNJ14-GB   .......... .......... .......... .......... .......... .. [226]
PUNJ16-GB   ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
PUNJ23-GB   .......... .......... .......... .......... .T........ T. [226]
PUNJ24-GB   .......... .......... .......... .......... .......... T. [226]
SK1-GB      ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
SK2-GB      ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
SK3-GB      ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
SK4-GB      ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
T064-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
T077-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
T107-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
T117-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
T124-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
T128-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
T131-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TC103-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TC22-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
TC28-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD183-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD201-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD207-GB    .......... .......... .......... .......... .......... .S [226]
TD29-GB     .......... .......... .......... .......... .......... .. [226]
TD297A-GB   ....K.E..D Q.K......N ...ET..... .......... .......... .. [226]
TD403-GB    .......... .......... .......... .......... .......... .. [226]
TD414-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD424-GB    .......... .......... ...ET..... .......... .......... .. [226]
TD425-GB    .......... .......... ...ET..... .......... .......... .S [226]
TD425B-GB   .......... .......... .......... .......... .......... .. [226]
TD430A-GB   .......... .......... .......... .......... .......... .. [226]
TD430B-GB   ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD438-GB    ....K.E..D Q.K.....QN .TKE...... .......... .......... .S [226]
TD439A-GB   ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
TD439B-GB   .......... .......... .......... .......... .......... .S [226]
TD452-GB    ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
TD458A-GB   ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TD458B-GB   .......... .......... .......... .......... .......... .. [226]
TE26-GB     .......... .......... ...ET..... .......... .......... .S [226]
TF127-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TF14-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TFF18-GB    ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TG40-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TG44-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
TG46-GB     ....K.E... .......... ...ET..... .......... .......... .. [226]
TG48-GB     ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
TG55-GB     ....K.E..D Q.K.....QN ..KET..... .......... .......... .. [226]
TG57-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
TUR1-GB     .......... .......... .......... .......... .......... .. [226]
TUR2-GB     .......... .......... .......... .......... .......... .. [226]
TUR3-GB     .......... ....Y..... ..Y..Q.E.. .......... .......... .. [226]
TUR4-GB     .......... ....Y..... .......... .......... .......... .. [226]
TUR5-GB     .......... .......... .......... .......... .......... .. [226]
TUR7-GB     .......... .......... .......... .......... .......... .. [226]
TUR8-GB     .......... .......... ..Y....... .......... .......... .. [226]
TV400-GB    ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
V100-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
VM278-GB    ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
VM55-GB     ....K.E..D Q.K.....QN ..KE...... .......... .......... .S [226]
A-P-ADM     .......... .......... .......... .......... .......... .. [226]
B-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
C-P-ADM     .......... .......... .......... .......... .......... .. [226]
D-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
E-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
F-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
G-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
H-P-ADM     .......... .......... .......... .......... .......... .. [226]
I-P-ADM     ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
J-P-ADM     .......... .......... .......... .......... .......... .. [226]
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K-P-ADM     .......... .......... .......... .......... .......... .. [226]
KB-P-ADM    .......... .......... .......... .......... .......... .. [226]
131-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
136-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
185-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
212-P-NOREL .......... .......... .......... .......... .......... .. [226]
321-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
340-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
442-P-NOREL ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
66-P-NOREL  .......... .......... .......... .......... .......... .. [226]
738-P-NOREL .......... .......... .......... .......... .......... .. [226]
827-P-NOREL .......... .......... .......... .......... .......... .. [226]
913-P-NOREL .......... .......... .......... .......... .......... .. [226]
103A-P-REL  .......... .......... .......... .......... A......... .. [226]
103B-P-REL  .......... .......... .......... .......... .......... .. [226]
126A-P-REL  .......... .......... .......... .......... .......... .. [226]
126B-P-REL  .......... .......... .......... .......... .......... .. [226]
216A-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
216B-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
243A-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
246A-P-REL  .......... .......... .......... .......... A......... .. [226]
246B-P-REL  .......... .......... .......... .......... .......... .. [226]
294A-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
294B-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
297A-P-REL  ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
361A-P-REL  .......P.. .......... .......... .......... .......... .. [226]
361B-P-REL  .......... .......... .......... .......... .......... .. [226]
370A-P-REL  .......... .......... .......... .......... .......... .. [226]
95A-P-REL   ....K.E..D Q.K.....QN ..KE...... .......... .......... .. [226]
95B-P-REL   .......... .......... .......... .......... .......... .. [226]
SALPAT-SAL  ....K.E..D Q.K.....QN ...ET..... .......... .......... .. [226]
YOELII-YO   ...IN.DA.S K.A.TSE.DK IRTLKR.S.. .V.LAI...M .T...L.E.I VD [226]
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Alignment 9: MSP-1 gene, nucleotide sequences from Manaus, reference sequences Salvador I (SALPAT) and Belém (BELEM, BELPAT) and sequences retrieved from Gene Bank. Belém was the reference strain, identical nucleotides are represented by dots, and gaps are represented by dashes.

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BELEM-BEL   ATT GAC AAG TTG AAG GAC TTC ATC CCC AAA ATC GAG AGC ATG ATC GCC ACT GAG AAG  [ 57]
BELPAT-BEL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BD1-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BD2-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BD4-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BD6-GB      ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
BD9-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP1-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP13-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP29-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP30-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP39-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BP63-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BR07-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
BR44-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
IN1-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
ITA1-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
ITA2-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
ITA3-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
ITA4-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
PUNJ14-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
PUNJ16-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
PUNJ23-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
PUNJ24-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SK1-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SK2-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SK3-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SK4-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
T077-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
T107-GB     ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
T117-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
T124-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
T128-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
T131-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TC103-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TC22-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TC28-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD183-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD201-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD207A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD207B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD29-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD403-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD414-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD424-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD425-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD425B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD430A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD430B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD438-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD439A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD439B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD452-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD458A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TD458B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TE26-GB     ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
TF127-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TF14-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TFF18-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TG40-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
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TG44-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TG46-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TG48-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TG55-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TG57-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TO64-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR1-GB      ... .C. GT. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR2-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR3-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR4-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR5-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR7-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TUR8-GB      ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
TV400-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
V100-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
VM278-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
VM55-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
A-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
B-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
C-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
D-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
E-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
F-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
G-P-ADM      ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
H-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
I-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
J-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
K-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
KB-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
131-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
136-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
185-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
212-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
321-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
340-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
442-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
66-P-NOREL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
738-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
827-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
913-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
103A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
103B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
126B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
216B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
243A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
246B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
294A-P-REL   ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
294B-P-REL   ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
297A-P-REL   ... ... ... ... ... ..T ..T ... ..A ... ..T C.. GC. ... ... A.. ... ... ...  [ 57]
361A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
361B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
370A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95A-P-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
95B-P-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
SALPAT-SAL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 57]
YOELII-YO    G.. ... .GA C.T GCC C.A ..T ..T ..A ... ... ..A ..T ... ..T ..T .AA ... ...  [ 57]
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Alignment 9 continuing

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BELEM-BEL    GCC AAG CCG GCA GCG TCA GCG CCA --- --- GTG ACC AGT GGA CAA TTG CTT AGA GGA  [114]
BELPAT-BEL   ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ... ...  [114]
BD1-GB       AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BD2-GB       AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BD4-GB       AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BD6-GB       ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
BD9-GB       AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP1-GB       AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP13-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP29-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP30-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP39-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
BP63-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
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BR07-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
BR44-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
IN1-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
ITA1-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
ITA2-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
ITA3-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
ITA4-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
PUNJ14-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
PUNJ16-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
PUNJ23-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
PUNJ24-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SK1-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SK2-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SK3-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SK4-GB      AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
T077-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
T107-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
T117-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
T124-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
T128-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
T131-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TC103-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TC22-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TC28-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD183-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD201-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD207A-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD207B-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD29-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD403-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD414-GB    A.. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD424-GB    .A. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD425-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD425B-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD430A-GB   A.. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD430B-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD438-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD439A-GB   ... ... ... A.. --- --- ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TD439B-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD452-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD458A-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TD458B-GB   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TE26-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TF127-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TF14-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TFF18-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TG40-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TG44-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TG46-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TG48-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TG55-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TG57-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TO64-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TUR1-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
TUR2-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TUR3-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TUR4-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TUR5-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TUR7-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TUR8-GB     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
TV400-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
V100-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
VM278-GB    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
VM55-GB     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
A-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
B-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
C-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
D-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
E-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
F-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
G-P-ADM     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
H-P-ADM     ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
I-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
J-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
K-P-ADM     AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
KB-P-ADM    AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
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131-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
136-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
185-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
212-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
321-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
340-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
442-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
66-P-NOREL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
738-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
827-P-NOREL AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
913-P-NOREL ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
103A-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
103B-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
126A-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
126B-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
216A-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
216B-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
243A-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
246A-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
246B-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
294A-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
294B-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
297A-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
361A-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
361B-P-REL  ... ... ... ... ... ... ... ... --- --- ... ... ... ... ... ... ... ... ...  [114]
370A-P-REL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
95A-P-REL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
95B-P-REL   AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
SALPAT-SAL  AA. ... ... A.C .T. G.. ... G.. GAT ATA ... G.A .AG ... ... .C. ... ... ...  [114]
YOELII-YO   .AA .GA AT. .A. CAA GG. C.T G.. ATT ACT .GA GAA TC. .A. G.. G.A .CA TCT ..C  [114]
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Alignment 9 continuing

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BELEM-BEL   TCA AGC GAA GCA GCG ACA GAG GTC ACA ACC AAT GCG GTA ACA TCT GAA GAT CAA CAA  [171]
BELPAT-BEL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
BD1-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BD2-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BD4-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BD6-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
BD9-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP1-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP13-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP29-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP30-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP39-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BP63-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
BR07-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
BR44-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
IN1-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
ITA1-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
ITA2-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
ITA3-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
ITA4-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
PUNJ14-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
PUNJ16-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
PUNJ23-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
PUNJ24-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SK1-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SK2-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SK3-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SK4-GB      G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
T077-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
T107-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
T117-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
T124-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
T128-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
T131-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TC103-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TC22-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TC28-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD183-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD201-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD207A-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD207B-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD29-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
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TD403-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD414-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD424-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD425-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD425B-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD430A-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD430B-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD438-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD439A-GB   ... ... ... CG. ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TD439B-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD452-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD458A-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TD458B-GB   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TE26-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TF127-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TF14-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TFF18-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TG40-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TG44-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TG46-GB     ... ... ... ... ... ... ... ... ... ... ... .G. ... ... ... ... .TA ... ...  [171]
TG48-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TG55-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TG57-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TO64-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TUR1-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
TUR2-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TUR3-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TUR4-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TUR5-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TUR7-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TUR8-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
TV400-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
V100-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
VM278-GB    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
VM55-GB     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
A-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
B-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
C-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
D-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
E-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
F-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
G-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
H-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
I-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
J-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
K-P-ADM     G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
KB-P-ADM    G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
131-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
136-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
185-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
212-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
321-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
340-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
442-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
66-P-NOREL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
738-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
827-P-NOREL G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
913-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
103A-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
103B-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
126A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
126B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
216A-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
216B-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
243A-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
246A-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
246B-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
294A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
294B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
297A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
361A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
361B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .TA ... ...  [171]
370A-P-REL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
95A-P-REL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
95B-P-REL   G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
SALPAT-SAL  G.. ..T ... A.. .G. ... ACT .G. .AT ..A GTC AAT .CG CA. A.A .CT .TA GT. ...  [171]
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YOELII-YO   C.T ..T .CT .A. T.A T.. ACA .AT .G. T.A .CA CAA TCT T.. A.A TCC TCA TCC TC.  [171]
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Alignment 9 continuing

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BELEM-BEL   CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAG CAA CAA CAG CAA CAA  [228]
BELPAT-BEL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
BD1-GB      ... ... ... ... ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BD2-GB      ... ... ... ... ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BD4-GB      --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BD6-GB      ... ... ... ... ..G ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
BD9-GB      ... ... ... ... ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BP1-GB      ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BP13-GB     .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BP29-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BP30-GB     ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BP39-GB     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
BP63-GB     .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
BR07-GB     ... ... ... ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- ---  [228]
BR44-GB     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
IN1-GB      ... .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
ITA1-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
ITA2-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
ITA3-GB     ... ... ... ... ... ... ... ... ..G ... ... ..G ... ..A ... ... --- --- ---  [228]
ITA4-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
PUNJ14-GB   ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
PUNJ16-GB   ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
PUNJ23-GB   ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
PUNJ24-GB   ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
SK1-GB      ... ... ... ... ..G ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [228]
SK2-GB      ... ... ... ... ..G ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [228]
SK3-GB      ... ... ... ... ..G ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [228]
SK4-GB      ... ... ... ... ..G ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [228]
T077-GB     ... ... ... ... ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
T107-GB     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
T117-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
T124-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
T128-GB     ... ... ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
T131-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TC103-GB    --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TC22-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TC28-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD183-GB    --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD201-GB    --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD207A-GB   --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD207B-GB   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
TD29-GB     ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TD403-GB    ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TD414-GB    ..T .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD424-GB    ... ... ... ..G ... ... ..G ... ... ..G ... ... ... ..A --- --- --- --- ---  [228]
TD425-GB    ... ... ... ..G ... ... ..G ... ... ..G ... ... ... ..A --- --- --- --- ---  [228]
TD425B-GB   ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TD430A-GB   ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TD430B-GB   --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD438-GB    ..T .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD439A-GB   ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- --- --- --- ---  [228]
TD439B-GB   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
TD452-GB    ... .C. ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TD458A-GB   --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TD458B-GB   ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TE26-GB     ... ... ... ... ... ... ... ..G ... ... ..G ... ... ... ... ... ... ... ...  [228]
TF127-GB    --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TF14-GB     --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TFF18-GB    --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TG40-GB     --- .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TG44-GB     --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TG46-GB     ... ... ... ... ... ... ... ... ... ... ... ..G ... ..A ... ... --- --- ---  [228]
TG48-GB     ... ... ... ... ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TG55-GB     --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TG57-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TO64-GB     --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
TUR1-GB     ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
TUR2-GB     ... ... ... ... ... ... ... ... ... ..G ... ... ..G ... ..A ... ... --- --- ---  [228]
TUR3-GB     ... ... ... ... ... ..G ... ... ..G ... ... ..G ... ..A ... ... --- --- ---  [228]
TUR4-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... --- --- --- --- --- ---  [228]
TUR5-GB     ... ... ... ... ... ..G ... ... ..G ... ... ..G ... ..A ... ... --- --- ---  [228]
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TUR7-GB      ... ... ... ... ... ..G ... ... ..G ... ... ..G ... ..A ... ... --- --- ---  [228]
TUR8-GB      ... ... ... ... ..G ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
TV400-GB     ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
V100-GB      --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
VM278-GB     ... ... ... ... ... ... ..G ... ... ..G ... ... ... ..A --- --- --- --- ---  [228]
VM55-GB      ... .C. ... ..T ... GT. GC. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
A-P-ADM      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
B-P-ADM      ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
C-P-ADM      ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
D-P-ADM      .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
E-P-ADM      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
F-P-ADM      .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
G-P-ADM      ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
H-P-ADM      ... ... ... ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- ---  [228]
I-P-ADM      ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
J-P-ADM      ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
K-P-ADM      ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
KB-P-ADM     ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
131-P-NOREL  ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
136-P-NOREL  ... ... ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
185-P-NOREL  ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
212-P-NOREL  ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
321-P-NOREL  .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
340-P-NOREL  ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
442-P-NOREL  ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
66-P-NOREL   ... ... ... ..G ... ... ..G ... ... ..G ... ... ..G ..A ... ... ..A --- ---  [228]
738-P-NOREL  ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
827-P-NOREL  ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
913-P-NOREL  ... ... ... ... ... ..G ... ... ..G ... ... ... ... --- --- --- --- --- ---  [228]
103A-P-REL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
103B-P-REL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
126A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
126B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
216A-P-REL   .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
216B-P-REL   .C. .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
243A-P-REL   ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
246A-P-REL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
246B-P-REL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
294A-P-REL   ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
294B-P-REL   ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
297A-P-REL   ... ... ... ... ... ... ... ..G ... ... ..G ... ... ..A ... --- --- --- ---  [228]
361A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
361B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ..A ... ...  [228]
370A-P-REL   ... ... ... ..G ... ... ..G ... ... ... ... ... ..G ..A ... ..G ..A ... ...  [228]
95A-P-REL    ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
95B-P-REL    ... ... ... ... ... ... ..G ... ... ..G ... ... ..G ..A ... ..G ..A ... ...  [228]
SALPAT-SAL   --- .C. ... ..T ... GT. GT. A.T GC. GT. ACG GT. ..G .CT GG. AC. ACA GG. ..T  [228]
YOELII-YO    TCC TC. TCT TC. ACC .C. GC. GC. GC. G.. TCC TCC TC. GCC AC. TT. .CA G.. GC.  [228]
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Alignment 9 continuing

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BELEM-BEL    CAA CAA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- TCA CAA GTA  [285]
BELPAT-BEL   ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
BD1-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BD2-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BD4-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BD6-GB       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
BD9-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BP1-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BP13-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BP29-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BP30-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BP39-GB      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
BP63-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
BR07-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
BR44-GB      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
IN1-GB       ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
ITA1-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
ITA2-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
ITA3-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
ITA4-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
PUNJ14-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
PUNJ16-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
PUNJ23-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAT ACA AAT TCA GTC CAA GCA G.. ... ..T  [285]
PUNJ24-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
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SK1-GB       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
SK2-GB       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
SK3-GB       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
SK4-GB       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
T077-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
T107-GB      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
T117-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
T124-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
T128-GB      ... GC. CAA GGT GGA GAA GCA GAA GCA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
T131-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TC103-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TC22-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TC28-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD183-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD201-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD207A-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD207B-GB    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD29-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD403-GB     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD414-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD424-GB     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD425-GB     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD425B-GB    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD430A-GB    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD430B-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD438-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD439A-GB    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD439B-GB    ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD452-GB     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TD458A-GB    ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TD458B-GB    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TE26-GB      ... ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TF127-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TF14-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TFF18-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TG40-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TG44-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TG46-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TG48-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TG55-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TG57-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TO64-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
TUR1-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR2-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR3-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR4-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR5-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR7-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TUR8-GB      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
TV400-GB     ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
V100-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
VM278-GB     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
VM55-GB      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
A-P-ADM      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
B-P-ADM      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
C-P-ADM      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
D-P-ADM      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
E-P-ADM      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
F-P-ADM      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
G-P-ADM      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
H-P-ADM      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
I-P-ADM      ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
J-P-ADM      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
K-P-ADM      ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
KB-P-ADM     ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
131-P-NOREL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
136-P-NOREL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
185-P-NOREL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
212-P-NOREL  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
321-P-NOREL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
340-P-NOREL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
442-P-NOREL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
66-P-NOREL   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
738-P-NOREL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
827-P-NOREL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
913-P-NOREL  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
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103A-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
103B-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
126A-P-REL  ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- --- ... ... ...  [285]
126B-P-REL  ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- --- ... ... ...  [285]
216A-P-REL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
216B-P-REL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
243A-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
246A-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
246B-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
294A-P-REL  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
294B-P-REL  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
297A-P-REL  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
361A-P-REL  ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- --- ... ... ...  [285]
361B-P-REL  ... ..G CAA CAA CAG CAA CAA CAA CAA --- --- --- --- --- --- --- --- --- ... ... ...  [285]
370A-P-REL  ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
95A-P-REL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- C..  [285]
95B-P-REL   ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... ... ...  [285]
SALPAT-SAL  ... GC. CAA GGT GGA GAA GCA GAA ACA CAA ACA AAT TCA GTA CAA GCA G.. ... ..T  [285]
YOELII-YO   .CC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [285]
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Alignment 9 continuing

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BELEM-BEL   GTA CCA GCA CCT GCA GGA GAT GCC CAA CAA GTA ATC TCA ACA CAA CCG ACT AGT CAA  [342]
BELPAT-BEL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
BD1-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BD2-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BD4-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BD6-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
BD9-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BP1-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BP13-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BP29-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BP30-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
BP39-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
BP63-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
BR07-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
BR44-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
IN1-GB      CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
ITA1-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
ITA2-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.C ... ..C ...  [342]
ITA3-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
ITA4-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
PUNJ14-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
PUNJ16-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
PUNJ23-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
PUNJ24-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
SK1-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
SK2-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
SK3-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
SK4-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
T077-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
T107-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
T117-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
T124-GB     CA. .A. A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
T128-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. .TC ...  [342]
T131-GB     CA. .A. A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TC103-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TC22-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TC28-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TD183-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TD201-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TD207A-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TD207B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD29-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD403-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD414-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ... ...  [342]
TD424-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD425-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD425B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD430A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD430B-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TD438-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TD439A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD439B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TD452-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
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TD458A-GB   CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TD458B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TE26-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TF127-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TF14-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TFF18-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TG40-GB     CA. .A. A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TG44-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TG46-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TG48-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TG55-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. .TC ...  [342]
TG57-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
TO64-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
TUR1-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR2-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR3-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR4-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR5-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR7-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TUR8-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
TV400-GB    CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
V100-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
VM278-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
VM55-GB     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
A-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
B-P-ADM     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
C-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
D-P-ADM     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..G ...  [342]
E-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
F-P-ADM     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
G-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
H-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
I-P-ADM     CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
J-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
K-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
KB-P-ADM    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
131-P-NOREL CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
136-P-NOREL CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
185-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
212-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
321-P-NOREL CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
340-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
442-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
66-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
738-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
827-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
913-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
103A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
103B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
126A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
126B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
216A-P-REL  CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
216B-P-REL  CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. ... ..C ...  [342]
243A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
246A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
246B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
294A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
294B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
297A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
361A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
361B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
370A-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
95A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
95B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
SALPAT-SAL  CA. .A. A.. ... ... ... .CG .G. GG. ..G ... GC. ... ... ... A.. .T. ..C ...  [342]
YOELII-YO   --- G.. C.. G.A .A. .C. .CA T.. .C. TC. AC. GAA G.. T.. G.. GAA ..A .CA AT.  [342]
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Alignment 9 continuing

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BELEM-BEL   TCC GCA GCA CCA GGC GTA TCA GCC ACA --- --- --- --- --- --- --- --- --- ---  [399]
BELPAT-BEL  ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
BD1-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BD2-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BD4-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
BD6-GB      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
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BD9-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BP1-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BP13-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BP29-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BP30-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
BP39-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
BP63-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
BR07-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
BR44-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
IN1-GB      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
ITA1-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCG GAA CCA GCA CCA GCC GTT --- --- ---  [399]
ITA2-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCG GAA CCA GCA CCA GCC GTC --- --- ---  [399]
ITA3-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
ITA4-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
PUNJ14-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
PUNJ16-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
PUNJ23-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
PUNJ24-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
SK1-GB      ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
SK2-GB      ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
SK3-GB      ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
SK4-GB      ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
T077-GB     G.. T.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
T107-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
T117-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
T124-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
T128-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
T131-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TC103-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
TC22-GB     G.. C.. ... ... AAT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TC28-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
TD183-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD201-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD207A-GB   G.. C.. ... ... AAT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD207B-GB   ... ... ... ... ..G ... ... ... ... CCA GCA CCA ACA CCT GCT GCC GCA GCC GCC  [399]
TD29-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD403-GB    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD414-GB    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD424-GB    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD425-GB    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD425B-GB   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD430A-GB   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD430B-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD438-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD439A-GB   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD439B-GB   ... ... ... ... ... ... ... ... ... CCA GCA CCA ACA CCT GCT GCC GCA GCC GCC  [399]
TD452-GB    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TD458A-GB   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TD458B-GB   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TE26-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TF127-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TF14-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TFF18-GB    G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TG40-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TG44-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TG46-GB     ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TG48-GB     G.. T.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
TG55-GB     G.. C.. ... ... AAT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TG57-GB     G.. C.. ... ... AAT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TO64-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
TUR1-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR2-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR3-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR4-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR5-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR7-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TUR8-GB     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
TV400-GB    G.. C.. ... ... AAT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
V100-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
VM278-GB    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
VM55-GB     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
A-P-ADM     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
B-P-ADM     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
C-P-ADM     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
D-P-ADM     G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
E-P-ADM     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
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F-P-ADM      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
G-P-ADM      ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
H-P-ADM      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
I-P-ADM      G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
J-P-ADM      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
K-P-ADM      ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
KB-P-ADM     ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
131-P-NOREL  G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
136-P-NOREL  G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
185-P-NOREL  ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
212-P-NOREL  ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
321-P-NOREL  G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
340-P-NOREL  ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
442-P-NOREL  ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
66-P-NOREL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
738-P-NOREL  ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
827-P-NOREL  ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
913-P-NOREL  ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
103A-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
103B-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
126A-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
126B-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
216A-P-REL   G.. C.. ... ... ACT CA. G.C T.. --- CAA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
216B-P-REL   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GTC --- --- ---  [399]
243A-P-REL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
246A-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
246B-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
294A-P-REL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
294B-P-REL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
297A-P-REL   ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
361A-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
361B-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
370A-P-REL   ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
95A-P-REL    ... ... ... ... .A. ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
95B-P-REL    ... ... ... ... ... ... ... ... ... --- --- --- --- --- --- --- --- --- ---  [399]
SALPAT-SAL   G.. C.. ... ... ACT CA. G.C T.. --- CCA GAA CCA GCA CCA GCC GCC --- --- ---  [399]
YOELII-YO    C.. C.T A.C A.. CAA .A. A.. CAA C.. --- --- --- --- --- --- --- --- --- ---  [399]
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Alignment 9 continuing

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BELEM-BEL    CCA GCA CCA ACA CCT GCT GCC GCA GCC GCC CCA GCA CCA GCC ATG TCC AAA CTG GAA  [456]
BELPAT-BEL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
BD1-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BD2-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BD4-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BD6-GB       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
BD9-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BP1-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BP13-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BP29-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BP30-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BP39-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
BP63-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
BR07-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
BR44-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
IN1-GB       ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... ... ... ... ... ... ...  [456]
ITA1-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... C.. ... ... ...  [456]
ITA2-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
ITA3-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
ITA4-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
PUNJ14-GB    ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
PUNJ16-GB    ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... C.. T.. ... ... ... .G.  [456]
PUNJ23-GB    ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... ... ... ... ... ... ...  [456]
PUNJ24-GB    ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
SK1-GB       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
SK2-GB       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
SK3-GB       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
SK4-GB       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
T077-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
T107-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
T117-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... ... ... ... ... ... ...  [456]
T124-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
T128-GB      ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ...  [456]
T131-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TC103-GB     ... C.. T.G ... ... ... ... ... .TT ..T ... ... ... ... ... ... ... ... ...  [456]
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TC22-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TC28-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... ... ... ... ... ... ... ...  [456]
TD183-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD201-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD207A-GB    ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD207B-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD29-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD403-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD414-GB     ... ... ... ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD424-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD425-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD425B-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD430A-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD430B-GB    ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD438-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD439A-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD439B-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD452-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TD458A-GB    ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TD458B-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TE26-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TF127-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TF14-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TFF18-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TG40-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TG44-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TG46-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TG48-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TG55-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TG57-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
TO64-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
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TUR2-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TUR3-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TUR4-GB      ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TUR5-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TUR7-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TUR8-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
TV400-GB     ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
V100-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
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VM55-GB      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
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C-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
D-P-ADM      ... C.. T.G ... ... ... ... ... ... .TT ..T ... ... ... A.. ... ... ... ... ... ...  [456]
E-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [456]
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Alignment 9 continuing

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TD458B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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TG48-GB     ..T ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
TG55-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
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VM278-GB    ..T ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
VM55-GB     ..T ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
A-P-ADM     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
B-P-ADM     ..T ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
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D-P-ADM     ..T ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
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212-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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340-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
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66-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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913-P-NOREL ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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103B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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126B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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246B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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294B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T  [513]
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361B-P-REL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [513]
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BD9-GB      ..T ..G ... ... ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ...  [570]
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ITA1-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [570]
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TF127-GB     ..T ... ..T ... ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... [570]
TF14-GB      ..T ... ..T ... ... ... ... A.A ... G.G ... ... G.T C.. ... A.. ... ... ... [570]
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Alignment 9 continuing

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Alignment 9 continuing

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TD439B-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
TD452-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TD458A-GB   ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TD458B-GB   ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TE26-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
TF127-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TF14-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TFF18-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TG40-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TG44-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
TG46-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TG48-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TG55-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TG57-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TO64-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
TUR1-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR2-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR3-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR4-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR5-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR7-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TUR8-GB     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
TV400-GB    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
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V100-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
VM278-GB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
VM55-GB      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. .. [680]
A-P-ADM      ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
B-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
C-P-ADM      ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
D-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
E-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
F-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
G-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
H-P-ADM      ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
I-P-ADM      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
J-P-ADM      ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
K-P-ADM      ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
KB-P-ADM     ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
131-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
136-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
185-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
212-P-NOREL  ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
321-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
340-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
442-P-NOREL  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
66-P-NOREL   ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
738-P-NOREL  ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
827-P-NOREL  ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
913-P-NOREL  ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
103A-P-REL   ..T ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
103B-P-REL   ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
126A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
126B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
216A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
216B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
243A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
246A-P-REL   ..T ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
246B-P-REL   ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
294A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
294B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
297A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
361A-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
361B-P-REL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
370A-P-REL   ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
95A-P-REL    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
95B-P-REL    ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. [680]
SALPAT-SAL   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... .. [680]
YOELII-YO    CAA A.T ..T .TG CCT A.T ATG TAT .CA CTT TAT G.A AGT ATA G.T GAT GGT TT [680]
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